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(57) Abstract

The invention relates generally to the changes in gene expression in ischemic heart tissue compared to normal human heart tissue. The invention relates specifically to a novel human gene which is expressed in ischemic human heart tissue.

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IDENTIFICATION OF A cDNA ASSOCIATED WITH ISCHEMIA IN HUMAN HEART TISSUE

FIELD OF THE INVENTION

The invention relates generally to the changes in gene expression in ischemic heart tissue compared to normal human heart tissue. The invention relates specifically to a novel human gene which is expressed in ischemic human heart tissue. This application is related to U.S. Provisional Application No. 60/098,683, filed September 1, 1998, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Cardiovascular disease is a general diagnostic category consisting of several separate diseases. Coronary heart disease and cerebrovascular disease are major components of cardiovascular disease with 478,530 dying of coronary heart disease and 144,070 dying of cerebrovascular disease in the U.S. in 1991. See Cecil Textbook of Medicine, Bennet and Plum Eds., W.B. Saunders Co., 1996. Many of the acute forms of coronary heart disease are caused by coronary artery abnormalities such as coronary atherosclerosis. Among the more common causes and contributing factors in sudden cardiac death are chronic ischemic heart disease and ischemic cardiomyopathy.

Chronic ischemic heart disease and ischemic cardiomyopathy are caused in part by episodes of insufficient myocardial oxygen supply. Myocardial oxygen supply is governed by coronary blood flow and the ability of the myocardium to extract oxygen from the blood delivered to it. Unlike other organs, the heart always extracts oxygen with near maximal efficiency from the blood. Even under situations of minimal demand, there is little potential for enhanced oxygen extraction to counter increased oxygen demands. Coronary blood flow, on the other hand, can increase several-fold in normal subjects as a result of coronary arterial vasodilation. Coronary arterial vasodilation is regulated by the coronary endothelium which releases vasodilatory

substances, most importantly nitric oxide.

In atherosclerotic coronary heart disease, endothelial dysfunction may diminish production of vasodilatory substances, such as nitric oxide. Myocardial ischemia results when autoregulatory vasodilation is prevented, whether by flow-limiting coronary arterial stenosis or by endothelial dysfunction. In both cases, arterial blood flow can no longer increase proportional to rising oxygen demands. In other situations, myocardial ischemia may occur when oxygen demands are constant but there is a primary decrease in coronary blood flow mediated via coronary artery spasm, rapid evolution of the underlying atherosclerotic plaque leading to a reduced coronary arterial lumen caliber, and/or intermittent microvascular plugging by platelet aggregates.

At the molecular level, ischemia is characterized by the differential expression of numerous genes compared to normal heart tissue. For instance, in human heart failure caused by ischemic cardiomyopathy, expression of the β_1- and β_2-adrenergic receptors of the adenyl cyclase signal transduction system is impaired by reductions in the expression of mRNA for each receptor (Ihl-Vahl *et al.*, *J Mol Cell Cardiol* **28**:1-10, 1996). Ischemic injury is also known to lead to the differential expression of heat shock and immediate early genes such as *hsp*70, *c-fos*, *c-jun*, *jun*-B as well the genes encoding angiotensin receptor subtypes (Plumier *et al.*, *J Mol Cell Cardiol* **28**:1251-1260, 1996; Wharton *et al.*, *J Pharmoc Experiment Therap* **284**(1) 323-336, 1998; and Heads *et al.*, *J Mol Cell Cardiol* **27**:2133-2148, 1995).

The identification of new genes that are differentially expressed in ischemic heart tissue will allow for the development of numerous diagnostic and therapeutic applications such as molecular probes and new agents which modulate the activity or expression of these genes.

SUMMARY OF THE INVENTION

The present invention is based on our discovery of a new gene which is expressed in ischemic heart tissue. The invention includes isolated nucleic acid molecules selected from the group consisting of an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, an isolated nucleic

acid molecule that encodes a fragment of at least 6 amino acids of SEQ ID No.2 or SEQ ID No.4, an isolated nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID No.1 or SEQ ID No.3 under conditions of sufficient stringency to produce a clear signal and an isolated nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4 under conditions of sufficient stringency to produce a clear signal.

The present invention further includes the nucleic acid molecules operably linked to one or more expression control elements, including vectors comprising the isolated nucleic acid molecules. The invention further includes host cells transformed to contain the nucleic acid molecules of the invention and methods for producing a protein comprising the step of culturing a host cell transformed with a nucleic acid molecule of the invention under conditions in which the protein is expressed.

The invention further provides an isolated polypeptide selected from the group consisting of an isolated polypeptide comprising the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising a fragment of at least 10 amino acids of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising conservative amino acid substitutions of SEQ ID No.2 or SEQ ID No.4 and naturally occurring amino acid sequence variants of SEQ ID No.2 or SEQ ID No.4.

The invention further provides an isolated antibody that binds to a polypeptide of the invention, including monoclonal and polyclonal antibodies.

The invention further provides methods of identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the steps of: exposing cells which express the nucleic acid to the agent; and determining whether the agent modulates expression of said nucleic acid, thereby identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4.

The invention further provides methods of identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the steps of: exposing cells which express the protein to the agent; and determining whether the agent modulates at least one activity of said protein, thereby

identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4. Moreover, assay methods are provided which comprise conditions which simulate physiological cardiac stresses, including simulation of ischemic conditions and occlusion of arteries.

The invention further provides methods of identifying binding partners for a protein comprising the sequence of SEQ ID No. 2 or SEQ ID No.4, comprising the steps of: exposing said protein to a potential binding partner; and determining if the potential binding partner binds to said protein, thereby identifying binding partners for a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

The present invention further provides methods of modulating the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the step of: administering an effective amount of an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4. The invention also provides methods of modulating at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the step of: administering an effective amount of an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

The present invention further provides for non-human transgenic animals comprising the nucleic acids of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Figure 1 is a Northern blot using a probe derived from SEQ ID No.1.

Figure 2A-B Figure 2 is a PCR quantification of clone 980 mRNA in normal and ischemic heart tissue compared to the differential display gel. Figure 2A is a section of the differential display gel. Figure 2B represents the normalized PCR results.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

I. General Description

The present invention is based in part on identifying a new gene that is expressed in human ischemic heart tissue. This gene encodes a protein predicted to consist of 339 amino acids.

The protein can serve as a target for agents that can be used to modulate the expression or activity of the protein. For example, agents may be identified which modulate biological processes associated with ischemic injury to the heart such as chronic ischemic heart disease and ischemic cardiomyopathy. Agents may also be identified which modulate the biological processes associated with recovery from ischemic injury to the heart.

The present invention is further based on the development of methods for isolating binding partners that bind to the protein. Probes based on the protein are used as capture probes to isolate potential binding partners, such as other proteins. Dominant negative proteins, DNAs encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into cells to affect function. Additionally, these proteins provide a novel target for screening of synthetic small molecules and combinatorial or naturally occurring compound libraries to discover novel therapeutics to regulate heart function.

II. Specific Embodiments

A. The Protein Associated with Ischemic Heart Tissue

The present invention provides isolated protein, allelic variants of the protein, and conservative amino acid substitutions of the protein. As used herein, the protein or polypeptide refers to a protein that has the human amino acid sequence depicted in SEQ ID No.2 or SEQ ID No.4. The invention includes naturally occurring allelic variants and proteins that have a slightly different amino acid sequence than that specifically recited above. Allelic variants, though possessing a slightly different amino acid sequence than those recited above, will still have the same or similar biological

functions associated with the 339 amino acid protein.

As used herein, the family of proteins related to the 339 amino acid protein refer to proteins that have been isolated from organisms in addition to humans. The methods used to identify and isolate other members of the family of proteins related to the 339 amino acid protein are described below.

The proteins of the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated protein.

The proteins of the present invention further include conservative variants of the proteins herein described. As used herein, a conservative variant refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the protein. A substitution, insertion or deletion is said to adversely affect the protein when the altered sequence prevents or disrupts a biological function associated with the protein. For example, the overall charge, structure or hydrophobic/hydrophilic properties of the protein can be altered without adversely affecting a biological activity. Accordingly, the amino acid sequence can be altered, for example to render the peptide more hydrophobic or hydrophilic, without adversely affecting the biological activities of the protein.

Ordinarily, the allelic variants, the conservative substitution variants, and the members of the protein family will have an amino acid sequence having at least 75% amino acid sequence identity with the human sequence set forth in SEQ ID No.2 or SEQ ID No.4, more preferably at least 80%, even more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to such sequences is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the known peptides, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. In a related aspect, conservative substitution refers to a substitution of one amino acid for another with

generally similar properties (size, hydrophobicity, charge, etc). N-terminal, C-terminal or internal extensions, deletions, or insertions into the peptide sequence shall not be construed as affecting homology.

Thus, the proteins of the present invention include molecules having the amino acid sequence disclosed in SEQ ID No.2 or SEQ ID No.4; fragments thereof having a consecutive sequence of at least about 3, 4, 5, 6, 10, 15, 20, 25, 30, 35 or more amino acid residues of the 339 amino acid protein; amino acid sequence variants of such sequence wherein an amino acid residue has been inserted N- or C-terminal to, or within, the disclosed sequence; and amino acid sequence variants of the disclosed sequence, or their fragments as defined above, that have been substituted by another residue. Contemplated variants further include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis, and the corresponding proteins of other animal species, including but not limited to rabbit, rat, murine, porcine, bovine, ovine, equine and non-human primate species, and the alleles or other naturally occurring variants of the family of proteins; and derivatives wherein the protein has been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope).

As described below, members of the family of proteins can be used: 1) to identify agents which modulate at least one activity of the protein; 2) in methods of identifying binding partners for the protein, 3) as an antigen to raise polyclonal or monoclonal antibodies, and 4) as a therapeutic agent.

B. Nucleic Acid Molecules

The present invention further provides nucleic acid molecules that encode the protein having SEQ ID No.2 or SEQ ID No.4 and the related proteins herein described, preferably in isolated form. As used herein, "nucleic acid" is defined as RNA or DNA that encodes a protein or peptide as defined above, or is complementary to nucleic acid sequence encoding such peptides, or hybridizes to such nucleic acid and remains stably bound to it under appropriate stringency conditions, or encodes a polypeptide sharing at

least 75% sequence identity, preferably at least 80%, and more preferably at least 85%, with the peptide sequences. Specifically contemplated are genomic DNA, cDNA, mRNA and antisense molecules, as well as nucleic acids based on alternative backbones or including alternative bases whether derived from natural sources or synthesized. Such hybridizing or complementary nucleic acids, however, are defined further as being novel and unobvious over any prior art nucleic acid including that which encodes, hybridizes under appropriate stringency conditions, or is complementary to nucleic acid encoding a protein according to the present invention.

Homology or identity is determined by **BLAST** (**B**asic **L**ocal **A**lignment **S**earch **T**ool) analysis using the algorithm employed by the programs **blastp, blastn, blastx, tblastn** and **tblastx** (Karlin, *et al., Proc Natl Acad Sci USA* **87**: 2264-2268, 1990 and Altschul, S. F., *J Mol Evol* **36**: 290-300, 1993, fully incorporated by reference) which are tailored for sequence similarity searching. The approach used by the **BLAST** program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.* (*Nature Genetics* **6**: 119-129, 1994) which is fully incorporated by reference. The search parameters for **histogram, descriptions, alignments, expect** (*i.e.*, the statistical significance threshold for reporting matches against database sequences), **cutoff, matrix** and **filter** are at the default settings. The default scoring matrix used by **blastp, blastx, tblastn**, and **tblastx** is the **BLOSUM62** matrix (Henikoff, *et al., Proc Natl Acad Sci* USA **89**: 10915-10919, 1992 fully incorporated by reference). For **blastn**, the scoring matrix is set by the ratios of **M** (i.e., the reward score for a pair of matching residues) to **N** (i.e., the penalty score for mismatching residues), wherein the default values for **M** and **N** are 5 and -4, respectively.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015M NaCl/0.0015M sodium titrate/0.1% SDS at 50°C., or (2) employ during hybridization a denaturing agent such as formamide, for

example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2 x SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal. For example, sufficient stringency conditions are contemplated such that target (e.g., SEQ ID NOs: 1 and 3) and closely related sequences (e.g., nucleic acids encoding SEQ ID NOs: 2 and 4 and variants) can be distinguished and isolated (see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed pp. 9.47-9.58; 11.1-11.19 and 11.45-11-57, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989 and Methods in Enzymology, Vol.**152**, (Berger *et al.*, eds), pp.399-407 and 620-622, Academic Press, Inc., New York 1987).

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid encoding other polypeptides from the source of nucleic acid.

The present invention further provides fragments of the encoding nucleic acid molecule. As used herein, a fragment of an encoding nucleic acid molecule refers to a small portion of the entire protein encoding sequence. The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein, the fragment will need to be large enough to encode the functional region(s) of the protein. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming.

Fragments of the encoding nucleic acid molecules of the present invention (*i.e.*, synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding proteins of the invention can easily be synthesized by chemical techniques, for example, the phosphotriester method of

Matteucci, *et al.*, (*J. Am. Chem. Soc.* 103:3185-3191, 1981) or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the gene, followed by ligation of oligonucleotides to build the complete modified gene.

The encoding nucleic acid molecules of the present invention may further be modified so as to contain a detectable label for diagnostic and probe purposes. A variety of such labels are known in the art and can readily be employed with the encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides and the like. A skilled artisan can employ any of the art known labels to obtain a labeled encoding nucleic acid molecule.

Modifications to the primary structure itself by deletion, addition, or alteration of the amino acids incorporated into the protein sequence during translation can be made without destroying the activity of the protein. Such substitutions or other alterations result in proteins having an amino acid sequence encoded by a nucleic acid falling within the contemplated scope of the present invention.

C. Isolation of Other Related Nucleic Acid Molecules

As described above, the identification of the human nucleic acid molecule having SEQ ID No.1 or SEQ ID No 4 allows a skilled artisan to isolate nucleic acid molecules that encode other members of the protein family in addition to the human sequence herein described. Further, the presently disclosed nucleic acid molecules allow a skilled artisan to isolate nucleic acid molecules that encode other members of the family of proteins in addition to the 339 amino acid protein having SEQ ID No.2 or SEQ ID No. 4.

Essentially, a skilled artisan can readily use the amino acid sequence of SEQ ID No.2 or SEQ ID No.4 to generate antibody probes to screen expression libraries prepared from appropriate cells. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe a mammalian cDNA or genomic expression library, such as lambda gtll library, to obtain the appropriate coding sequence for other members of the protein family.

The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructions using control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the coding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the protein family from any mammalian organism. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives.

Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively clone an encoding nucleic acid molecule. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other encoding nucleic acid molecules.

D. rDNA molecules Containing a Nucleic Acid Molecule

The present invention further provides recombinant DNA molecules (rDNAs) that contain a coding sequence. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in situ*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al., Molecular Cloning* (1989). In the preferred rDNA molecules, a coding DNA sequence is operably linked to expression control sequences and/or vector sequences.

The choice of vector and/or expression control sequences to which one of the protein family encoding sequences of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of the structural gene included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other

regulatory elements. Preferably, the inducible promoter is readily controlled, such as being responsive to a nutrient in the host cell's medium.

In one embodiment, the vector containing a coding nucleic acid molecule will include a prokaryotic replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or bacteriophage promoter capable of directing the expression (transcription and translation) of the coding gene sequences in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA), pPL and pKK223 available from Pharmacia, Piscataway, N.J.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to form a rDNA molecules that contains a coding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are pSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1 (ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the

gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (*neo*) gene. (Southern *et al.*, *J. Mol. Anal.Genet* 1:327-341, 1982.) Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by co-transfection of the host cell, and selected by culturing in the appropriate drug for the selectable marker.

E. Host Cells Containing an Exogenously Supplied Coding Nucleic Acid Molecule

The present invention further provides host cells transformed with a nucleic acid molecule that encodes a protein of the present invention. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a protein of the invention are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of the gene product. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, baby hamster kidney cells (BHK), and the like eukaryotic tissue culture cell lines.

Any prokaryotic host can be used to express a rDNA molecule encoding a protein of the invention. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with a rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen *et al.*, *Proc. Natl. Acad. Sci. USA* 69:2110, 1972; and Maniatis *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed, see, for example, Graham *et al.*, *Virol* 52:456, 1973; Wigler *et al.*, *Proc Natl Acad Sci USA* 76:1373-

76, 1979.

Successfully transformed cells, i.e., cells that contain a rDNA molecule of the present invention, can be identified by well known techniques including the selection for a selectable marker. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J Mol Biol* **98**:503, 1975, or Berent *et al.*, *Biotech.* **3**:208, 1985 or the proteins produced from the cell assayed via an immunological method.

F. Production of Recombinant Proteins using a rDNA Molecule

The present invention further provides methods for producing a protein of the invention using nucleic acid molecules herein described. In general terms, the production of a recombinant form of a protein typically involves the following steps:

First, a nucleic acid molecule is obtained that encodes a protein of the invention, such as the nucleic acid molecule depicted in SEQ ID No.1 or SEQ ID NO.3, nucleotides 184-1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3. If the encoding sequence is uninterrupted by introns, it is directly suitable for expression in any host.

The nucleic acid molecule is then preferably placed in operable linkage with suitable control sequences, as described above, to form an expression unit containing the protein open reading frame. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the recombinant protein. Optionally the recombinant protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in appropriate hosts. The construction of expression vectors that are operable in a variety of hosts is accomplished using appropriate replicons and control sequences, as set forth above. The control sequences, expression vectors, and transformation methods are dependent on the

type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with the nucleic acid molecules of the invention to produce recombinant protein.

G. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for use in isolating and identifying binding partners of proteins of the invention In detail, a protein of the invention is mixed with a potential binding partner or an extract or fraction of a cell under conditions that allow the association of potential binding partners with the protein of the invention. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with a protein of the invention are separated from the mixture. The binding partner that bound to the protein of the invention can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance the entire 339 amino acid protein of SEQ ID No.2 or SEQ ID No. 4 can be used. Alternatively, a fragment of the protein can be used.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell. The preferred source of cellular extracts will be cells derived from human heart tissue, for instance, ischemic human heart tissue. Alternatively, cellular extracts may be prepared from normal human heart tissue or available cell lines, particularly heart or muscle derived cell lines.

A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the protein of the invention under conditions in which association of the protein with the binding partner

can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins. Alternatively, binding partners may be identified using a Far-Western assay according to the procedures of Takayama *et al., Methods Mol Biol* **69**:171-84, 1997 or Sauder *et al., J GenVirol* 77(5):991-6, 1996 or identified through the use of epitope tagged proteins or GST fusion proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

H. Methods to Identify Agents that Modulate the Expression a Nucleic Acid Encoding the Ischemic Heart Associated Protein.

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding a protein of the invention such as a protein having the amino acid sequence of SEQ ID No.2 or SEQ ID No.4. Such assays may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid of the invention, for instance a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4, if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

In one assay format, cell lines that contain reporter gene fusions between the open reading frame defined by nucleotides 184-1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3 and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.* (1990) *Anal Biochem* **188**:245-254). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4.

Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding a protein of the invention such as the protein having SEQ ID No.2 or SEQ ID No.4. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press, 1989).

In order to assay gene expression of the present invention in a physiologically relevant manner, tissues may be analyzed under conditions which model physiological

cardiac cell stimuli. For example, some model systems simply include substrate depletion and increased intracellular acidity (Ch'en *et al., Prog Biophys Mol Biol* 69(2-3):515-38, 1998). Others are more complex. For example, Wilders *et al.* used isolated guinea pig ventricular myocytes which were electrically coupled via a coupling-clamp circuit to a comprehensive computer model of a guinea pig ventricular myocyte to assess alterations in the critical value of coupling conductance required for action potential conduction from the real cells to the model cell when the real cells are exposed to a solution that simulates acute ischemia (Wilders *et al., Circulation* 30;99(12):1623-9, 1999). Further, exposure of myocytes to tumor necrosis factor-alpha, IL-1beta, or lipopolysaccharide has been shown to simulate effects seen during reprofusion injury (Gwechenberger *et al., Circulation* 99(4):546-51, 1999). Further, models have been developed to simulate ischemia and reprofusion in quiescent human ventricular cardiomyocytes. Cellular injury and metabolic parameters can be assessed after various interventions, such as: preconditioning cells with anoxia, hypoxia, anoxic supernatants, or hypoxic supernatants (Cohen *et al., Circulation* 98(19 Suppl):II184-94; discussion II194-6, 1998). Another model is hypoxia-reoxygenation stress in the rat myoblast cell line, H9c2, which simulates ischemic preconditioning in heart tissue (Sakamoto *et al., Biochem Biophys Res Commun* 20;251(2):576-9, 1998).

In a preferred embodiment, assays which incubate cells under conditions that simulate cardiac ischemia and/or heart stress *in vitro* include, but are not limited to, for example, fluid shear stress in human endothelial cells (Houston *et al., Artherioscler Thromb Vacs Biol* 19(2):281-289, 1999) and passive stretch of cultured myocytes (Yamazaki *et al., J Mol Cel Cardiol* 27(1):133-140, 1995). "Shear stress" refers to the energy necessary produce an opposite but parallel sliding motion across a body's plane. In a related aspect, "shear stress" refers to substantially the physiological equivalent pressure produced in various tissues or organs such as force present in the vasculature by the actions of cardiac muscle. In a related aspect, assays which simulate ischemia by stressing the heart *in vivo* include, but are not limited to, for example, occlusion of the heart by ligation of blood vessels in animal models (Soloman *et al., J Am Coll Cardiol* 33(3): 854-856, 1999 and Kirma *et al., Jpn Circ J* 62(4):294-298, 1998).

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, NY, 1989) or Ausubel *et al.* (Current Protocols in Molecular Biology, Greene Publishing Co., NY, 1995).

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.* and Ausubel *et al.* as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support and the solid support exposed to at least one probe comprising at least one, or part of one of the sequences of the invention under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a porous glass wafer. The glass wafer can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid encoding

the protein having the sequence of SEQ ID No.2 or SEQ ID No.4 are identified.

Hybridization for qualitative and quantitative analysis of mRNAs may also be carried out by using a RNase Protection Assay (*i.e.*, RPA, see Ma *et al.*, *Methods* **10**: 273-238, 1996). Briefly, an expression vehicle comprising cDNA encoding the gene product and a phage specific DNA dependent RNA polymerase promoter (e.g., T7, T3 or SP6 RNA polymerase) is linearized at the 3' end of the cDNA molecule, downstream from the phage promoter, wherein such a linearized molecule is subsequently used as a template for synthesis of a labeled antisense transcript of the cDNA by *in vitro* transcription. The labeled transcript is then hybridized to a mixture of isolated RNA (*i.e.*, total or fractionated mRNA) by incubation at 45°C overnight in a buffer comprising 80% formamide, 40 mM Pipes, pH 6.4, 0.4 M NaCl and 1 mM EDTA. The resulting hybrids are then digested in a buffer comprising 40 µg/ml ribonuclease A and 2 µg/ml ribonuclease. After deactivation and extraction of extraneous proteins, the samples are loaded onto urea/polyacrylamide gels for analysis.

In another assay format, agents which effect the expression of the instant gene products, cells or cell lines would first be identified which express said gene products physiologically (*e.g.*, see example Figure 1 for tissue distribution via Northern blot, however, RPAs may serve the identical purpose of expression selection). Cell and/or cell lines so identified would be expected to comprise the necessary cellular machinery such that the fidelity of modulation of the transcriptional apparatus is maintained with regard to exogenous contact of agent with appropriate surface transduction mechanisms and/or the cytosolic cascades. Further, such cells or cell lines would be transduced or transfected with an expression vehicle (*e.g.*, a plasmid or viral vector) construct comprising an operable non-translated 5'-promoter containing end of the structural gene encoding the instant gene products fused to one or more antigenic fragments, which are peculiar to the instant gene products, wherein said fragments are under the transcriptional control of said promoter and are expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides or may further comprise an immunologically distinct tag. Such a process is well known in the art (see Maniatis, 1982).

Cells or cell lines transduced or transfected as outlined above would then be contacted with agents under appropriate conditions; for example, the agent comprises a pharmaceutically acceptable excipient and is contacted with cells comprised in an aqueous physiological buffer such as phosphate buffered saline (PBS) at physiological pH, Eagles balanced salt solution (BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and/or serum incubated at 37° C. Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells will be disrupted and the polypeptides of the disruptate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody to be further processed by immunological assay (e.g., ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the "agent contacted" sample will be compared with a control sample where only the excipient is contacted with the cells and an increase or decrease in the immunologically generated signal from the "agent contacted" sample compared to the control will be used to distinguish the effectiveness of the agent.

I. Methods to Identify Agents that Modulate at Least One Activity of the Ischemic Heart Associated Protein.

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of a protein of the invention such as the protein having the amino acid sequence of SEQ ID No.2 or SEQ ID No.4. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

In one format, the relative amounts of a protein of the invention between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptides. polypeptides or proteins of the invention if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the amino or carboxy terminus with a Cys residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein (*Nature* **256**(5517):495-7, 1975; *Eur J Immunol* **6**(7):511-9, 1976; and *Biotechnology* **24**:524-6, 1992)or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten. polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of $F(ab')_2$ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole

immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin, for instance, humanized antibodies.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of the a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis which takes into account the sequence of the target site and/or its conformation in connection with the agent's action. As described in the Examples, there is a proposed tyrosine kinase phoshporylation site in the protein having SEQ ID No.2 or SEQ ID No.4. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to the putative tyrosine kinase phosphorylation site at amino acid 98 of SEQ ID No.1.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive with critical positions of proteins of the invention. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies.

J. Uses for Agents that Modulate at Least One Activity of the Ischemic Heart Associated Protein.

As provided in the Examples, the proteins and nucleic acids of the invention, such as the protein having the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, are expressed in ischemic heart tissue. Agents that modulate or down-regulate the expression of the protein or agents such as agonists or antagonists of at least one activity of the protein may be used to modulate biological and pathologic processes associated with the protein's function and activity.

As used herein, a subject can be any mammal, so long as the mammal is in need of modulation of a pathological or biological process mediated by a protein of the invention. The term "mammal" is meant an individual belonging to the class Mammalia. The invention is particularly useful in the treatment of human subjects.

Pathological processes refer to a category of biological processes which produce a deleterious effect. For example, expression of a protein of the invention may be associated with chronic ischemic heart disease and ischemic cardiomyopathy. As used herein, an agent is said to modulate a pathological process when the agent reduces the degree or severity of the process. For instance, chronic ischemic heart disease or ischemic cardiomyopathy may be prevented or disease progression modulated after an ischemic event by the administration of agents which reduce or modulate in some way the expression or at least one activity of a protein of the invention.

The agents of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. For example, an agent of the present invention can be administered in combination with anti-thrombotic agents. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a

fashion such that the agents will act at the same time.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The present invention further provides compositions containing one or more agents which modulate expression or at least one activity of a protein of the invention. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise 0.1 to 100 μg/kg body wt. The preferred dosages comprise 0.1 to 10 μg/kg body wt. The most preferred dosages comprise 0.1 to 1 μg/kg body wt.

In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin

capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions according to generally accepted medical practice, such as anticoagulant agents, thrombolytic agents, or other antithrombotics, including platelet aggregation inhibitors, tissue plasminogen activators, urokinase, prourokinase, streptokinase, heparin, aspirin, or warfarin. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

K. Transgenic Animals

Transgenic animals containing mutant, knock-out or modified genes corresponding to the cDNA sequence of SEQ ID NO:1 or SEQ ID NO: 3 are also included in the invention. Transgenic animals are genetically modified animals into which recombinant, exogenous or cloned genetic material has been experimentally transferred. Such genetic material is often referred to as a "transgene". The nucleic acid sequence of the transgene, in this case a form of SEQ ID NO: 1 or SEQ ID NO: 3, may be integrated either at a locus of a genome where that particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal.

The term "germ cell line transgenic animal" refers to a transgenic animal in which the genetic alteration or genetic information was introduced into a germ line cell, thereby conferring the ability of the transgenic animal to transfer the genetic information to offspring. If such offspring in fact possess some or all of that alteration or genetic information, then they too are transgenic animals.

The alteration or genetic information may be foreign to the species of animal to

which the recipient belongs, foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

Transgenic animals can be produced by a variety of different methods including transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection (*see, e.g.*, U.S. Patent No. 4,736,866; U.S. Patent No. 5,602,307; Mullins *et al.*, *Hypertension* **22**(4):630-633, 1993; Brenin *et al.*, *Surg Oncol* **6**(2)99-110, 1997; Tuan (ed.), Recombinant Gene Expression Protocols, Methods in Molecular Biology, 1997, No. 62, Humana Press).

A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express simian SV 40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.*, *Genetics* **143**(4):1753-1760, 1996; or, are capable of generating a fully human antibody response (McCarthy, *The Lancet* **349**(9049):405, 1997).

While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, pigs, dogs, cats, monkeys, chimpanzees, hamsters, rabbits, cows and guinea pigs (*see, e.g.*, Kim *et al.*, *Mol Reprod Dev* **46**(4):515-526, 1997; Houdebine, *Reprod Nutr Dev* **35**(6):609-617, 1995; Petters *Reprod Fertil Dev* **6**(5):643-645, 1994 ; Schnieke *et al.*, *Science* **278**(5346):2130-2133, 1997; and Amoah *J Animal Science* **75**(2):578-585, 1997).

The method of introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method which favors co-transformation of

multiple nucleic acid molecules. Detailed procedures for producing transgenic animals are readily available to one skilled in the art, including the disclosures in U.S. Patent No. 5,489,743 and U.S. Patent No. 5,602,307.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1

Identification of Differentially Expressed Ischemic Heart mRNA

Heart tissue was obtained from five male patients with inotrope-dependent post-ischemic cardiomyopathy exhibiting severe myocyte and or cardiac hypertrophy with at least three years since their first myocardial infarction. Heart tissue was also obtained from 5 female patients with idiopathic dilated cardiomyopathy exhibiting severe myocyte and/or cardiac hypertrophy and CHF duration of at least 2 years.

Total cellular RNA was prepared from the heart tissue described above as well as from control, non-ischemic heart tissue using the procedure of Newburger *et al.*, *J. Biol. Chem.* **266**(24):16171-7, 1981 and Newburger *et al.*, *Proc Natl Acad Sci USA* **85**:5215-5219, 1988.

Synthesis of cDNA was performed as previously described by Prashar *et al.* in WO 97/05286 and in Prashar *et al., Proc Natl Acad Sci. USA* **93**:659-663, 1996. Briefly, cDNA was synthesized according to the protocol described in the GIBCO/BRL kit for cDNA synthesis. The reaction mixture for first-strand synthesis included 6 μg of total RNA, and 200 ng of a mixture of 1-base anchored oligo(dT) primers with all three possible anchored bases (ACGTAATACGACTCACTATAGGGCGAATTGGGTCGACTTTTTTTTTTTTTTT Tn1 wherein n1=A/C or G) (SEQ ID NO:5) along with other components for first-strand synthesis reaction except reverse transcriptase. This mixture was incubated at 65°C for 5m, chilled on ice and the process repeated. Alternatively, the reaction mixture may include 10μg of total RNA, and 2 pmol of 1 of the 2-base anchored oligo(dT) primers a heel such as RP5.0 (CTCTCAAGGATCTTACCGCTT $_{18}$AT) (SEQ ID NO:6), or RP6.0 (TAATACCGCGCCACATAGCAT $_{18}$CG) (SEQ ID NO:7), or RP9.2 (CAGGGTAGACGACGCTACGCT$_{18}$GA) (SEQ ID NO: 8) along with other components for first-strand synthesis reaction except reverse transcriptase. This mixture

was then layered with mineral oil and incubated at 65°C for 7 min followed by 50°C for another 7 min. At this stage, 2μl of Superscript reverse transcriptase (200 units/μl; GIBCO/BRL) was added quickly and mixed, and the reaction continued for 1 hr at 45-50°C. Second-strand synthesis was performed at 16°C for 2 hr. At the end of the reaction, the cDNAs were precipitated with ethanol and the yield of cDNA was calculated. In our experiments, ≈200 ng of cDNA was obtained from 10μg of total RNA.

The adapter oligonucleotide sequences were

A1 (TAGCGTCCGGCGCAGCGACGGCCAG) (SEQ ID NO:9)and

A2 (GATCCTGGCCGTCGGCTGTCTGTCGGCGC) (SEQ ID NO:10). One microgram of oligonucleotide A2 was first phosphorylated at the 5′ end using T4 polynucleotide kinase (PNK). After phosphorylation, PNK was heated denatured, and 1μg of the oligonucleotide A1 was added along with 10× annealing buffer (1 M NaCl/100 mM Tris-HCl, pH8.0/10 mM EDTA, pH8.0) in a final vol of 20 μl. This mixture was then heated at 65°C for 10 min followed by slow cooling to room temperature for 30 min, resulting in formation of the Y adapter at a final concentration of 100 ng/μl. About 20 ng of the cDNA was digested with 4 units of *Bgl* II in a final vol of 10 μl for 30 min at 37°C. Two microliters (≈4 ng of digested cDNA) of this reaction mixture was then used for ligation to 100 ng (≈50-fold) of the Y-shaped adapter in a final vol of 5μl for 16 hr at 15°C. After ligation, the reaction mixture was diluted with water to a final vol of 80 μl (adapter ligated cDNA concentration, ≈50 pg/μl) and heated at 65°C for 10 min to denature T4 DNA ligase, and 2-μl aliquots (with ≈100 pg of cDNA) were used for PCR.

The following sets of primers were used for PCR amplification of the adapter ligated 3′ -end cDNAs:

TGAAGCCGAGACGTCGGTGG$(T)_{18}$ n1, n2 (SEQ ID NO:11) (wherein n1, n2 = AA, AC, AG AT CA CC CG CT GA GC GG and GT) as the 3' primer with A1 as the 5' primer or alternatively

RP 5.0, RP 6.0, or RP 9.2 used as 3′ primers with primer A1.1 serving as the 5′ primer. To detect the PCR products on the display gel, 24 pmol of oligonucleotide A1

or A1.1 was 5′ -end-labeled using 15 μl of [γ-^{32} P]ATP (Amersham; 3000 Ci/mmol) and PNK in a final volume of 20 μl for 30 min at 37°C. After heat denaturing PNK at 65°C for 20 min, the labeled oligonucleotide was diluted to a final concentration of 2 μM in 80 μl with unlabeled oligonucleotide A1.1. The PCR mixture (20μl) consisted of 2 μl (≈100 pg) of the template, 2μl of 10× PCR buffer (100 mM Tris·HCl, pH 8.3/500 mM KCl), 2 μl of 15 mM $MgCl_2$ to yield 1.5 mM final Mg^{2+} concentration optimum in the reaction mixture, 200 μM dNTPs, 200 nM each 5′ and 3′ PCR primers, and 1 unit of Amplitaq Gold®. Primers and dNTPs were added after preheating the reaction mixture containing the rest of the components at 85°C. This "hot start" PCR was done to avoid artefactual amplification arising out of arbitrary annealing of PCR primers at lower temperature during transition from room temperature to 94°C in the first PCR cycle. PCR consisted of 5 cycles of 94°C for 30 sec, 55°C for 2 min, and 72°C for 60 sec followed by 25 cycles of 94°C for 30 sec, 60°C for 2 min, and 72°C for 60 sec. A higher number of cycles resulted in smeary gel patterns. PCR products (2.5μl) were analyzed on 6% polyacrylamide sequencing gel. For double or multiple digestion following adapter ligation, 13.2 μl of the ligated cDNA sample was digested with a secondary restriction enzyme(s) in a final vol of 20 μl. From this solution, 3μl was used as template for PCR. This template vol of 3 μl carried ≈ 100 pg of the cDNA and 10 mM $MgCl_2$ (from the 10× enzyme buffer), which diluted to the optimum of 1.5 mM in the final PCR vol of 20 μl. Since Mg^{2+} comes from the restriction enzyme buffer, it was not included in the reaction mixture when amplifying secondarily cut cDNA. Individual cDNA fragments corresponding to mRNA species were separated by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography. Bands were extracted from the display gels as described by Liang *et al.* (*Cur Opin Immunol* 7:274-280, 1995), reamplified using the 5′ and 3′ primers, and subcloned into pCR-Script with high efficiency using the PCR-Script cloning kit from Stratagene. Plasmids were sequenced by cycle sequencing on an ABI automated sequencer. Alternatively, bands were extracted (cored) from the display gels, PCR amplified and sequenced directly without subcloning.

Fragment 3145 is a band that corresponds to a cDNA derived from a mRNA species that is expressed in at least one ischemic heart tissue sample. The band corresponding to fragment 3145 was sequenced. The sequence of the band is: ccaggagctatgaatgactcagtggtggaaatgcccttctggaaactgaatattaccttctgtaggaaaaggtggaaaataagc atctagaaggttgttgtgaatgactctgtgctggcaaaaatgcttgaaacctctatatttctttcgttcataagacgtaaaggtcaaat ttttcaagaaaagtcttttaataacaaaagcatgcagttctctgtgaaatctcaaatattgttgtaatagtctgtttcaatcttaaaaaga atcaataaaaacaaacaagggaaaa (SEQ ID NO:12).

Example 2

Cloning of a Full Length cDNA Corresponding to 3145

The full length cDNA corresponding to band 3145 was obtained by the oligo-pulling method. Briefly, a gene-specific oligo was designed based on cDNA fragment 3145. The oligo was labeled with biotin and used to hybridize with 2 ug of single strand plasmid DNA (cDNA recombinants) from a human heart cDNA library following the procedures of Sambrook *et al.*. The hybridized cDNAs were separated by streptavidin-conjugated beads and eluted by heating. The eluted cDNA was converted to double strand plasmid DNA and used to transform *E. coli* cells (DH10B) and the longest cDNA was screened. After confirmed by PCR using gene-specific primers, the cDNA clone was subjected to DNA sequencing.

The nucleotide sequence of the full-length cDNA corresponding to the differentially regulated band 3145 band is set forth in SEQ ID No:1 (PGP-980.1). The cDNA comprises 2008 base pairs with an open reading frame encoding a protein predicted to contain 339 amino acids. The predicted amino acid sequence is presented in SEQ ID Nos. 1 and 2.

The nucleotide sequence of a second full length cDNA corresponding to the differentially regulated band 3145 is set forth in SEQ ID NO:3 (PGP980.2). The cDNA comprises 1981 base pairs with an open reading frame encoding a protein predicted to contain 339 amino acids. The predicted amino acid sequence is set forth in SEQ ID

Nos: 3 and 4. The 339 amino acid sequence of PGP980.1 and PGP980.2 differ only by the presence of a cysteine residue at amino acid residue 66 in PGP980.1 and an arginine at the same amino acid residue in PGP980.2.

The predicted isoelectric point of the 2 proteins is approximately 7.52. Both proteins contain a putative signal sequence comprising amino acids 1-29.

Example 3

Northern blot and PCR Expression Analysis

The tissue distribution of RNA encoding the differentially regulated gene encoding the protein of SEQ ID NO:2 was analyzed by Northern blot as well as PCR expression analysis of RNA isolated from various tissues. RNA was isolated from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas using standard procedures. Northern blots were prepared using a probe derived from SEQ ID NO:1 with hybridization conditions as described by Sambrook *et al* (1989). PCR expression analysis was also performed using primers derived from SEQ ID NO:1 using AmpliTaq Gold PCR® amplification kits (Perkin Elmer). Figure 1 is a Northern blot demonstrating the presence of variable levels of specific RNA in all tissues.

Quantitative PCR Analysis of Expression Levels

Figure 2 is a PCR analysis of expression levels in normal and ischemic heart tissue samples compared to the detected levels in the differential display. Samples 206 and 558 are normal heart tissue samples. Samples 146, 149, 294, 320 and 327 are ischemic heart tissue samples (see Table 1). The first fragment in the top row of Fgure 2 is a differential display band from a normal heart tissue samples. Bands 2-6 correspond to the differential display bands for samples 146, 149, 294, 320 and 327, respectively.

Real time PCR detection was accomplished by the use of the ABI PRISM 7700 Sequence Detection System. The 7700 measures the fluorescence intensity of the sample each cycle and is able to detect the presence of specific amplicons within the PCR reaction. Each sample was assayed for the level of GAPDH and Clone 980.

GAPDH detection was performed using Perkin Elmer part#402869 according to the manufacturer's directions. Primers were designed for clone 980 using Primer Express, a program developed by PE to efficiently find primers and probes for specific sequences. These primers were used in conjunction with SYBR green (Molecular Probes), a nonspecific double stranded DNA dye, to measure the expression level of a clone 980, which was normalized to the GAPDH level in each sample. The normalized values are reported in Figure 2. Samples 206 and 558 are normal samples, the remainder are from ischemic male patients.

GL Sample	File #	Age	Gender	Pt Wt	Pt Ht	Heart Wt	Etiology
S00146A	20	66	Male	70	175	600	ischemic
S00149A	26	65	Male	94	178	724	ischemic
S00294A	73	66	Male	86	169	519	ischemic
S00320A	109	60	Male	82	168	562	ischemic
S00327A	116	67	Male	71	183	619	ischemic

Example 4

Method of Screening for Modulators of PGP980.1 and PGP980.2 Expression Using Shear-Stress Assay

Using human epithelial cells, a shear-stress of 1.5 N/m^2 is applied to cells in culture according to the method of Houston *et al.* (*Artherioscler Thromb Vacs Biol* 19(2):281-289, 1999). At specific time points during applied stress, candidate agents and diluent (*i.e.*, carrier minus agent; control) are contacted with human epithelial cells. Cells are removed and lysed in an appropriate buffer for isolation of total and/or messenger RNA in a similar fashion as described in Sambrook *et al.* (1989). Isolated nucleic acids are then assayed by a transcriptional profiling means to determine whether the candidate agent modulates the induction of PGP980.1 and PGP980.2. Agents which up- or down-regulate the expression of either one or both transcripts are then designated as modulators of PGP980.1 and PGP980.2.

Example 5

Method of Screening for Modulators of Myocardial PGP980.1 and PGP980.2 Expression Using Passive Stretch of Cardiomyocytes

Using cultured myocytes on silicone membranes, cells are passively stretched according to the method of Yamazaki *et al.* (*J Mol Cell Cardiol* 27(1):133-140, 1995). At specific time points during applied stress, candidate agents and diluent (*i.e.*, carrier minus agent; control) are contacted with human epithelial cells. Control and test cells are removed and lysed in an appropriate buffer for isolation of total and/or messenger RNA in a similar fashion as described in Sambrook *et al* (1989). Isolated nucleic acids are then assayed by a transcriptional profiling assay to determine whether the candidate agent modulates the induction of PGP980.1 and PGP980.2. Agents which up- or down-regulate the expression of either one or both transcripts will then be designated as modulators of PGP980.1 and PGP980.2.

Example 6

Method of Screening for Modulators of Myocardial PGP980.1 and PGP980.2

Expression Using an Animal Model for Occlusion and Reprofusion of the Heart

Animal models for occlusion of the heart are well documented (Soloman *et al., J Am Coll Cardiol* **33**(3): 854-856, 1999 and Kirma *et al., Jpn Circ J* **62**(4):294-298, 1998). For example, pigs are used wherein regional ischemia is produced in control and candidate agent treated animals by partially occluding (ligating) the left anterior descending coronary artery. After obtaining the baseline values for evaluation of heart rate, transmitral flow and blood pressure, agents are administered to the animals (including carrier-only for controls) and at various time points and/or after administration of various concentrations of candidate agents using a single time point, post occlusion, the hearts of the animals are removed for isolation of nucleic acids by standard methods as described in Sambrook *et al* (1989). Isolated nucleic acids are then assayed by a transcriptional profiling assay to determine whether the candidate agent modulates the induction of PGP980.1 and PGP980.2. Agents which up- or down-regulate of either one or both transcripts will then be designated as modulators of PGP980.1 and PGP980.2.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents and publications referred to in this application are herein incorporated by reference in their entirety.

WHAT IS CLAIMED:

1. An isolated nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4; (b) an isolated nucleic acid molecule that encodes a fragment of at least 6 amino acids of SEQ ID No.2 or SEQ ID No.4; (c) an isolated nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule comprising SEQ ID No.1 or SEQ ID No.3 under conditions of sufficient stringency to produce a clear signal; and (d) an isolated nucleic acid molecule which hybridizes to a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4 under conditions of sufficient stringency to produce a clear signal.

2. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises the sequence of SEQ ID No.1 or SEQ ID No.3.

3. The isolated nucleic acid molecule of claim 2, wherein the nucleic acid molecule consists of the sequence of SEQ ID No.1 or SEQ ID No.3.

4. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises nucleotides 184 to 1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleic acid molecule consists of nucleotides 184 to 1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3.

6. The isolated nucleic acid molecule of any one of claims 1-5, wherein said nucleic acid molecule is operably linked to one or more expression control elements.

7. A vector comprising an isolated nucleic acid molecule of any one of claims 1-5.

8. A host cell transformed to contain the nucleic acid molecule of any one claims 1-5.

9. A host cell comprising a vector of claim 7.

10. A host cell of claim 9, wherein said host is selected from the group consisting of prokaryotic hosts and eukaryotic hosts.

11. A method for producing a polypeptide comprising the step of culturing a host cell transformed with the nucleic acid molecule of any one of claims 1-5 under conditions in which the protein encoded by said nucleic acid molecule is expressed.

12. The method of claim 11, wherein said host cell is selected from the group consisting of prokaryotic hosts and eukaryotic hosts.

13. An isolated polypeptide produced by the method of claim 11.

14. An isolated polypeptide selected from the group consisting of an isolated polypeptide comprising the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising a fragment of at least 6 amino acids of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising conservative amino acid substitutions of SEQ ID No.2 or SEQ ID No.4 and naturally occurring amino acid sequence variants of SEQ ID No.2 or SEQ ID No.4.

15. An isolated antibody that binds to a polypeptide of either claim 13 or 14.

16. The antibody of claim 14 wherein said antibody is a monoclonal or polyclonal antibody.

17. A method of identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the steps of:

exposing cells which express the nucleic acid to the agent; and

determining whether the agent modulates expression of said nucleic acid, thereby identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4.

18. A method of identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the steps of:

exposing cells which express the protein to the agent;

determining whether the agent modulates at least one activity of said protein, thereby identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

19. The method of claim 19, wherein the agent modulates the phosphorylation of the protein.

20. A method of identifying binding partners for a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the steps of:

exposing said protein to a potential binding partner; and

determining if the potential binding partner binds to said protein, thereby identifying binding partners for a protein comprising the sequence of SEQ ID No 2 or SEQ ID No.4.

21. A method of modulating the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the step of:

administering an effective amount of an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID

No.4.

22. A method of modulating at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the step of:

administering an effective amount of an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

23. A method of screening for agents which modulate PGP980.1 gene expression comprising the steps:

a) incubating cells under conditions which simulate heart stress or cardiac ischemia in the presence or absence of an agent;

b) determining whether PGP980.1 is up- or down-regulated in the presence of the agent, wherein up- or down-regulation of PGP980.1 identifies the agent as a modulator.

24. A method of screening for agents which modulate PGP980.2. gene expression comprising the steps:

a) incubating cells under conditions which simulate heart stress or cardiac ischemia in the presence or absence of an agent;

b) determining whether PGP980.2. is up- or down regulated in the presence of the agent, wherein up- or down-regulation of PGP980.2. induction identifies the agent as a modulator.

25. The method of claim 23, wherein the incubation conditions comprise shear stress.

26. The method of claim 23, wherein the incubation conditions comprise passive stretch of myocytes.

27. The method of claim 24, wherein the incubation conditions comprise shear stress.

28. The method of claim 24, wherein the incubation conditions comprise passive stretch of myocytes.

29. A method of screening for agents which modulate PGP980.1 gene expression comprising the steps:

a) occluding blood vessels leading to the heart of test and control animals to produce conditions which simulate cardiac ischemia and

b) determining whether PGP980.1 is up-or down-regulated in the presence of an administered agent, wherein up or down regulation of PGP980.1 identifies the agent as a modulator.

30. A method of screening for agents which modulate PGP980.2. gene expression comprising the steps:

a) occluding blood vessels leading to the heart of test and control animals to produce conditions which simulate cardiac ischemia and

b) determining whether PGP980.2. is up-or down-regulated in the presence of an administered agent, wherein up-or down-regulation of PGP980.2. identifies the agent as a modulator.

31. A non-human transgenic animal comprising a nucleic acid molecule of any of claims 1-6.

32. A non-human transgenic animal which does not express a nucleic acid molecule of any of claims 1-6.

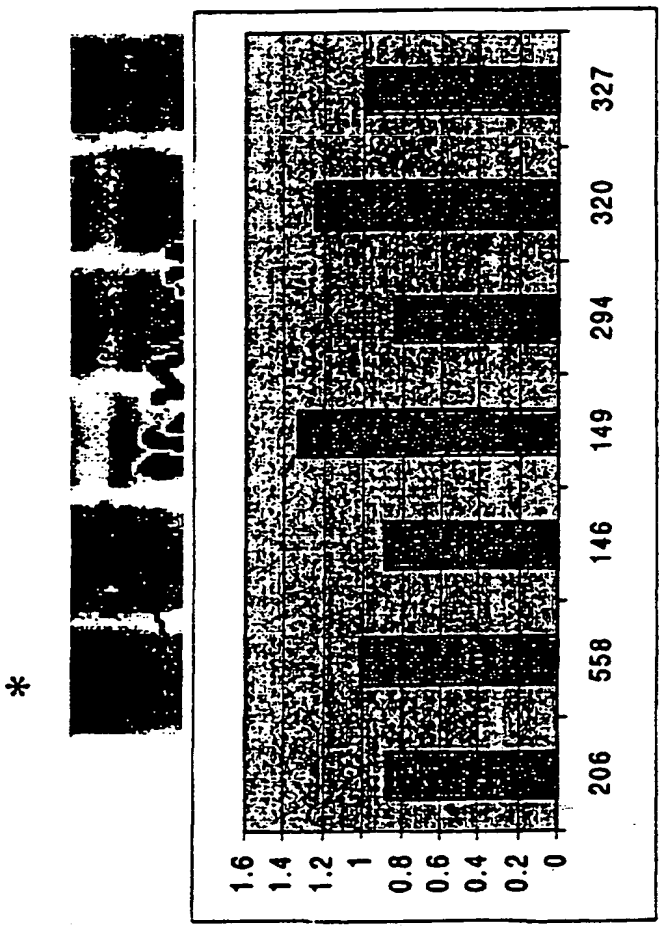

Fig. 2

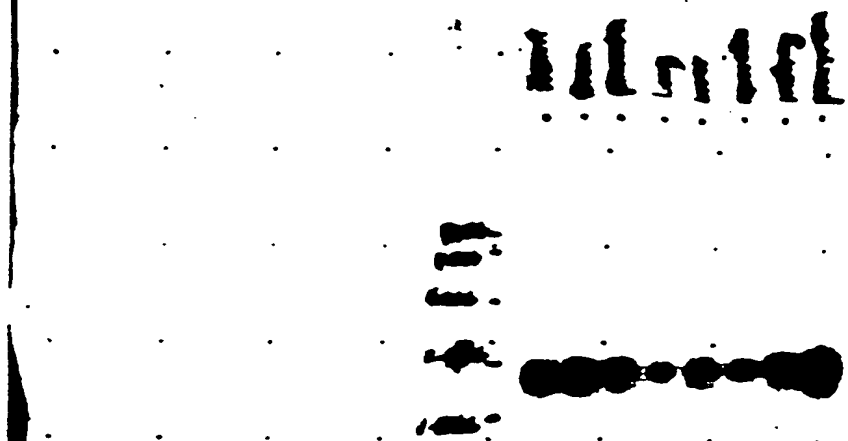

Fig. 1

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Einstein, Richard

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product of PGP 980 gene

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20015

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/8; 514/1; 536/23.5; 435/320.1, 325, 252.1, 326, 6, 7.1, 375; 530/350, 387.1,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank, Accession No. AA001371, HILLIER L et al. 'The WashU-Merck Project', abstract, unpublished, 29 November 1996. See sequence comparison.	1
X, P --- Y, P	WO 98/46757 A2 (GENETICS INSTITUTE, INC.) 22 October 1998, see entire document. Also see sequence comparison with N_Geneseq Accession No v62752.	1-14 --- 15-32
X, P --- Y, P	WO 98/54206 A1 (HUMAN GENOME SCIENCES, INC.) 03 December 1998, see entire document. Also see sequence comparison with N_Geneseq Accession No V08330.	1-16 --- 17-32
X	Database GenBank, Accession No. AA148037, HILLIER et al., 'Generation and analysis of 280,000 human expressed sequence tags', abstract, 05 December 1996, Genome Res. Vol. 6, No. 9, pages 807-828, see sequence comparison.	1

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RAM R. SHUKLA Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20015

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CAMERON, E. R. Recent advances in transgenic technology. Molecular Biotechnology. 1997, Vol 7, pages 253-265, see entire document.	31-32

INTERNATIONAL SEARCH REPORT

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PCT/US99/20015

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

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A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

800/8; 514/1; 536/23.5; 435/320.1, 325, 252.1, 326, 6, 7.1, 375; 530/350, 387.1,

CORRECTED VERSION*

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(72) Inventor; and
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(74) Agent: ADLER, Reid, G.; Morgan, Lewis & Bockius, LLP, 1800 M Street, N.W., Washington, DC 20036–5869 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: IDENTIFICATION OF A cDNA ASSOCIATED WITH ISCHEMIA IN HUMAN HEART TISSUE

(57) Abstract

The invention relates generally to the changes in gene expression in ischemic heart tissue compared to normal human heart tissue. The invention relates specifically to a novel human gene which is expressed in ischemic human heart tissue.

*(Referred to in PCT Gazette No. 40/2000, Section II)

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IDENTIFICATION OF A cDNA ASSOCIATED WITH ISCHEMIA IN HUMAN HEART TISSUE

FIELD OF THE INVENTION

The invention relates generally to the changes in gene expression in ischemic heart tissue compared to normal human heart tissue. The invention relates specifically to a novel human gene which is expressed in ischemic human heart tissue. This application is related to U.S. Provisional Application No. 60/098,683, filed September 1, 1998, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Cardiovascular disease is a general diagnostic category consisting of several separate diseases. Coronary heart disease and cerebrovascular disease are major components of cardiovascular disease with 478,530 dying of coronary heart disease and 144,070 dying of cerebrovascular disease in the U.S. in 1991. See Cecil Textbook of Medicine, Bennet and Plum Eds., W.B. Saunders Co., 1996. Many of the acute forms of coronary heart disease are caused by coronary artery abnormalities such as coronary atherosclerosis. Among the more common causes and contributing factors in sudden cardiac death are chronic ischemic heart disease and ischemic cardiomyopathy.

Chronic ischemic heart disease and ischemic cardiomyopathy are caused in part by episodes of insufficient myocardial oxygen supply. Myocardial oxygen supply is governed by coronary blood flow and the ability of the myocardium to extract oxygen from the blood delivered to it. Unlike other organs, the heart always extracts oxygen with near maximal efficiency from the blood. Even under situations of minimal demand, there is little potential for enhanced oxygen extraction to counter increased oxygen demands. Coronary blood flow, on the other hand, can increase several-fold in normal subjects as a result of coronary arterial vasodilation. Coronary arterial vasodilation is regulated by the coronary endothelium which releases vasodilatory

substances, most importantly nitric oxide.

In atherosclerotic coronary heart disease, endothelial dysfunction may diminish production of vasodilatory substances, such as nitric oxide. Myocardial ischemia results when autoregulatory vasodilation is prevented, whether by flow-limiting coronary arterial stenosis or by endothelial dysfunction. In both cases, arterial blood flow can no longer increase proportional to rising oxygen demands. In other situations, myocardial ischemia may occur when oxygen demands are constant but there is a primary decrease in coronary blood flow mediated via coronary artery spasm, rapid evolution of the underlying atherosclerotic plaque leading to a reduced coronary arterial lumen caliber, and/or intermittent microvascular plugging by platelet aggregates.

At the molecular level, ischemia is characterized by the differential expression of numerous genes compared to normal heart tissue. For instance, in human heart failure caused by ischemic cardiomyopathy, expression of the β_1- and β_2-adrenergic receptors of the adenyl cyclase signal transduction system is impaired by reductions in the expression of mRNA for each receptor (Ihl-Vahl *et al.*, *J Mol Cell Cardiol* **28**:1-10, 1996). Ischemic injury is also known to lead to the differential expression of heat shock and immediate early genes such as *hsp*70, *c-fos*, *c-jun*, *jun*-B as well the genes encoding angiotensin receptor subtypes (Plumier *et al.*, *J Mol Cell Cardiol* **28**:1251-1260, 1996; Wharton *et al.*, *J Pharmoc Experiment Therap* **284**(1) 323-336, 1998; and Heads *et al.*, *J Mol Cell Cardiol* **27**:2133-2148, 1995).

The identification of new genes that are differentially expressed in ischemic heart tissue will allow for the development of numerous diagnostic and therapeutic applications such as molecular probes and new agents which modulate the activity or expression of these genes.

SUMMARY OF THE INVENTION

The present invention is based on our discovery of a new gene which is expressed in ischemic heart tissue. The invention includes isolated nucleic acid molecules selected from the group consisting of an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, an isolated nucleic

acid molecule that encodes a fragment of at least 6 amino acids of SEQ ID No.2 or SEQ ID No.4, an isolated nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID No.1 or SEQ ID No.3 under conditions of sufficient stringency to produce a clear signal and an isolated nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4 under conditions of sufficient stringency to produce a clear signal.

The present invention further includes the nucleic acid molecules operably linked to one or more expression control elements, including vectors comprising the isolated nucleic acid molecules. The invention further includes host cells transformed to contain the nucleic acid molecules of the invention and methods for producing a protein comprising the step of culturing a host cell transformed with a nucleic acid molecule of the invention under conditions in which the protein is expressed.

The invention further provides an isolated polypeptide selected from the group consisting of an isolated polypeptide comprising the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising a fragment of at least 10 amino acids of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising conservative amino acid substitutions of SEQ ID No.2 or SEQ ID No.4 and naturally occurring amino acid sequence variants of SEQ ID No.2 or SEQ ID No.4.

The invention further provides an isolated antibody that binds to a polypeptide of the invention, including monoclonal and polyclonal antibodies.

The invention further provides methods of identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the steps of: exposing cells which express the nucleic acid to the agent; and determining whether the agent modulates expression of said nucleic acid, thereby identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4.

The invention further provides methods of identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the steps of: exposing cells which express the protein to the agent; and determining whether the agent modulates at least one activity of said protein, thereby

identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4. Moreover, assay methods are provided which comprise conditions which simulate physiological cardiac stresses, including simulation of ischemic conditions and occlusion of arteries.

The invention further provides methods of identifying binding partners for a protein comprising the sequence of SEQ ID No. 2 or SEQ ID No.4, comprising the steps of: exposing said protein to a potential binding partner; and determining if the potential binding partner binds to said protein, thereby identifying binding partners for a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

The present invention further provides methods of modulating the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the step of: administering an effective amount of an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4. The invention also provides methods of modulating at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the step of: administering an effective amount of an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

The present invention further provides for non-human transgenic animals comprising the nucleic acids of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Figure 1 is a Northern blot using a probe derived from SEQ ID No.1.

Figure 2A-B Figure 2 is a PCR quantification of clone 980 mRNA in normal and ischemic heart tissue compared to the differential display gel. Figure 2A is a section of the differential display gel. Figure 2B represents the normalized PCR results.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

I. General Description

The present invention is based in part on identifying a new gene that is expressed in human ischemic heart tissue. This gene encodes a protein predicted to consist of 339 amino acids.

The protein can serve as a target for agents that can be used to modulate the expression or activity of the protein. For example, agents may be identified which modulate biological processes associated with ischemic injury to the heart such as chronic ischemic heart disease and ischemic cardiomyopathy. Agents may also be identified which modulate the biological processes associated with recovery from ischemic injury to the heart.

The present invention is further based on the development of methods for isolating binding partners that bind to the protein. Probes based on the protein are used as capture probes to isolate potential binding partners, such as other proteins. Dominant negative proteins, DNAs encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into cells to affect function. Additionally, these proteins provide a novel target for screening of synthetic small molecules and combinatorial or naturally occurring compound libraries to discover novel therapeutics to regulate heart function.

II. Specific Embodiments

A. The Protein Associated with Ischemic Heart Tissue

The present invention provides isolated protein, allelic variants of the protein, and conservative amino acid substitutions of the protein. As used herein, the protein or polypeptide refers to a protein that has the human amino acid sequence depicted in SEQ ID No.2 or SEQ ID No.4. The invention includes naturally occurring allelic variants and proteins that have a slightly different amino acid sequence than that specifically recited above. Allelic variants, though possessing a slightly different amino acid sequence than those recited above, will still have the same or similar biological

functions associated with the 339 amino acid protein.

As used herein, the family of proteins related to the 339 amino acid protein refer to proteins that have been isolated from organisms in addition to humans. The methods used to identify and isolate other members of the family of proteins related to the 339 amino acid protein are described below.

The proteins of the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated protein.

The proteins of the present invention further include conservative variants of the proteins herein described. As used herein, a conservative variant refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the protein. A substitution, insertion or deletion is said to adversely affect the protein when the altered sequence prevents or disrupts a biological function associated with the protein. For example, the overall charge, structure or hydrophobic/hydrophilic properties of the protein can be altered without adversely affecting a biological activity. Accordingly, the amino acid sequence can be altered, for example to render the peptide more hydrophobic or hydrophilic, without adversely affecting the biological activities of the protein.

Ordinarily, the allelic variants, the conservative substitution variants, and the members of the protein family will have an amino acid sequence having at least 75% amino acid sequence identity with the human sequence set forth in SEQ ID No.2 or SEQ ID No.4, more preferably at least 80%, even more preferably at least 90%, and most preferably at least 95%. Identity or homology with respect to such sequences is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the known peptides, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. In a related aspect, conservative substitution refers to a substitution of one amino acid for another with

generally similar properties (size, hydrophobicity, charge, etc). N-terminal, C-terminal or internal extensions, deletions, or insertions into the peptide sequence shall not be construed as affecting homology.

Thus, the proteins of the present invention include molecules having the amino acid sequence disclosed in SEQ ID No.2 or SEQ ID No.4; fragments thereof having a consecutive sequence of at least about 3, 4, 5, 6, 10, 15, 20, 25, 30, 35 or more amino acid residues of the 339 amino acid protein; amino acid sequence variants of such sequence wherein an amino acid residue has been inserted N- or C-terminal to, or within, the disclosed sequence; and amino acid sequence variants of the disclosed sequence, or their fragments as defined above, that have been substituted by another residue. Contemplated variants further include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis, and the corresponding proteins of other animal species, including but not limited to rabbit, rat, murine, porcine, bovine, ovine, equine and non-human primate species, and the alleles or other naturally occurring variants of the family of proteins; and derivatives wherein the protein has been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope).

As described below, members of the family of proteins can be used: 1) to identify agents which modulate at least one activity of the protein; 2) in methods of identifying binding partners for the protein, 3) as an antigen to raise polyclonal or monoclonal antibodies, and 4) as a therapeutic agent.

B. Nucleic Acid Molecules

The present invention further provides nucleic acid molecules that encode the protein having SEQ ID No.2 or SEQ ID No.4 and the related proteins herein described, preferably in isolated form. As used herein, "nucleic acid" is defined as RNA or DNA that encodes a protein or peptide as defined above, or is complementary to nucleic acid sequence encoding such peptides, or hybridizes to such nucleic acid and remains stably bound to it under appropriate stringency conditions, or encodes a polypeptide sharing at

least 75% sequence identity, preferably at least 80%, and more preferably at least 85%, with the peptide sequences. Specifically contemplated are genomic DNA, cDNA, mRNA and antisense molecules, as well as nucleic acids based on alternative backbones or including alternative bases whether derived from natural sources or synthesized. Such hybridizing or complementary nucleic acids, however, are defined further as being novel and unobvious over any prior art nucleic acid including that which encodes, hybridizes under appropriate stringency conditions, or is complementary to nucleic acid encoding a protein according to the present invention.

Homology or identity is determined by **BLAST** (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs **blastp, blastn, blastx, tblastn** and **tblastx** (Karlin, *et al., Proc Natl Acad Sci USA* **87**: 2264-2268, 1990 and Altschul, S. F., *J Mol Evol* **36**: 290-300, 1993, fully incorporated by reference) which are tailored for sequence similarity searching. The approach used by the **BLAST** program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.* (*Nature Genetics* **6**: 119-129, 1994) which is fully incorporated by reference. The search parameters for **histogram, descriptions, alignments, expect** (*i.e.*, the statistical significance threshold for reporting matches against database sequences), **cutoff, matrix** and **filter** are at the default settings. The default scoring matrix used by **blastp, blastx, tblastn**, and **tblastx** is the **BLOSUM62** matrix (Henikoff, *et al., Proc Natl Acad Sci* USA **89**: 10915-10919, 1992 fully incorporated by reference). For **blastn**, the scoring matrix is set by the ratios of **M** (i.e., the reward score for a pair of matching residues) to **N** (i.e., the penalty score for mismatching residues), wherein the default values for **M** and **N** are 5 and -4, respectively.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015M NaCl/0.0015M sodium titrate/0.1% SDS at 50°C., or (2) employ during hybridization a denaturing agent such as formamide, for

example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2 x SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal. For example, sufficient stringency conditions are contemplated such that target (e.g., SEQ ID NOs: 1 and 3) and closely related sequences (e.g., nucleic acids encoding SEQ ID NOs: 2 and 4 and variants) can be distinguished and isolated (see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed pp. 9.47-9.58; 11.1-11.19 and 11.45-11-57, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989 and Methods in Enzymology, Vol.152, (Berger *et al.*, eds), pp.399-407 and 620-622, Academic Press, Inc., New York 1987).

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid encoding other polypeptides from the source of nucleic acid.

The present invention further provides fragments of the encoding nucleic acid molecule. As used herein, a fragment of an encoding nucleic acid molecule refers to a small portion of the entire protein encoding sequence. The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein, the fragment will need to be large enough to encode the functional region(s) of the protein. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming.

Fragments of the encoding nucleic acid molecules of the present invention (*i.e.*, synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding proteins of the invention can easily be synthesized by chemical techniques, for example, the phosphotriester method of

Matteucci, *et al.*, (*J. Am. Chem. Soc.* **103**:3185-3191, 1981) or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the gene, followed by ligation of oligonucleotides to build the complete modified gene.

The encoding nucleic acid molecules of the present invention may further be modified so as to contain a detectable label for diagnostic and probe purposes. A variety of such labels are known in the art and can readily be employed with the encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides and the like. A skilled artisan can employ any of the art known labels to obtain a labeled encoding nucleic acid molecule.

Modifications to the primary structure itself by deletion, addition, or alteration of the amino acids incorporated into the protein sequence during translation can be made without destroying the activity of the protein. Such substitutions or other alterations result in proteins having an amino acid sequence encoded by a nucleic acid falling within the contemplated scope of the present invention.

C. Isolation of Other Related Nucleic Acid Molecules

As described above, the identification of the human nucleic acid molecule having SEQ ID No.1 or SEQ ID No 4 allows a skilled artisan to isolate nucleic acid molecules that encode other members of the protein family in addition to the human sequence herein described. Further, the presently disclosed nucleic acid molecules allow a skilled artisan to isolate nucleic acid molecules that encode other members of the family of proteins in addition to the 339 amino acid protein having SEQ ID No.2 or SEQ ID No. 4.

Essentially, a skilled artisan can readily use the amino acid sequence of SEQ ID No.2 or SEQ ID No.4 to generate antibody probes to screen expression libraries prepared from appropriate cells. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe a mammalian cDNA or genomic expression library, such as lambda gtll library, to obtain the appropriate coding sequence for other members of the protein family.

The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructions using control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the coding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the protein family from any mammalian organism. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives.

Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively clone an encoding nucleic acid molecule. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other encoding nucleic acid molecules.

D. rDNA molecules Containing a Nucleic Acid Molecule

The present invention further provides recombinant DNA molecules (rDNAs) that contain a coding sequence. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in situ*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al., Molecular Cloning* (1989). In the preferred rDNA molecules, a coding DNA sequence is operably linked to expression control sequences and/or vector sequences.

The choice of vector and/or expression control sequences to which one of the protein family encoding sequences of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of the structural gene included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other

regulatory elements. Preferably, the inducible promoter is readily controlled, such as being responsive to a nutrient in the host cell's medium.

In one embodiment, the vector containing a coding nucleic acid molecule will include a prokaryotic replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or bacteriophage promoter capable of directing the expression (transcription and translation) of the coding gene sequences in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA), pPL and pKK223 available from Pharmacia, Piscataway, N.J.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to form a rDNA molecules that contains a coding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are pSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1 (ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the

gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (*neo*) gene. (Southern *et al.*, *J. Mol. Anal.Genet* 1:327-341, 1982.) Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by co-transfection of the host cell, and selected by culturing in the appropriate drug for the selectable marker.

E. Host Cells Containing an Exogenously Supplied Coding Nucleic Acid Molecule

The present invention further provides host cells transformed with a nucleic acid molecule that encodes a protein of the present invention. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a protein of the invention are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of the gene product. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, baby hamster kidney cells (BHK), and the like eukaryotic tissue culture cell lines.

Any prokaryotic host can be used to express a rDNA molecule encoding a protein of the invention. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with a rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen *et al.*, *Proc. Natl. Acad. Sci. USA* 69:2110, 1972; and Maniatis *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed, see, for example, Graham *et al.*, *Virol* **52**:456, 1973; Wigler *et al.*, *Proc Natl Acad Sci USA* **76**:1373-

76, 1979.

Successfully transformed cells, i.e., cells that contain a rDNA molecule of the present invention, can be identified by well known techniques including the selection for a selectable marker. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J Mol Biol* 98:503, 1975, or Berent *et al.*, *Biotech.* 3:208, 1985 or the proteins produced from the cell assayed via an immunological method.

F. Production of Recombinant Proteins using a rDNA Molecule

The present invention further provides methods for producing a protein of the invention using nucleic acid molecules herein described. In general terms, the production of a recombinant form of a protein typically involves the following steps:

First, a nucleic acid molecule is obtained that encodes a protein of the invention, such as the nucleic acid molecule depicted in SEQ ID No.1 or SEQ ID NO.3, nucleotides 184-1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3. If the encoding sequence is uninterrupted by introns, it is directly suitable for expression in any host.

The nucleic acid molecule is then preferably placed in operable linkage with suitable control sequences, as described above, to form an expression unit containing the protein open reading frame. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the recombinant protein. Optionally the recombinant protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in appropriate hosts. The construction of expression vectors that are operable in a variety of hosts is accomplished using appropriate replicons and control sequences, as set forth above. The control sequences, expression vectors, and transformation methods are dependent on the

type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with the nucleic acid molecules of the invention to produce recombinant protein.

G. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for use in isolating and identifying binding partners of proteins of the invention In detail, a protein of the invention is mixed with a potential binding partner or an extract or fraction of a cell under conditions that allow the association of potential binding partners with the protein of the invention. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with a protein of the invention are separated from the mixture. The binding partner that bound to the protein of the invention can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance the entire 339 amino acid protein of SEQ ID No.2 or SEQ ID No. 4 can be used. Alternatively, a fragment of the protein can be used.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell. The preferred source of cellular extracts will be cells derived from human heart tissue, for instance, ischemic human heart tissue. Alternatively, cellular extracts may be prepared from normal human heart tissue or available cell lines, particularly heart or muscle derived cell lines.

A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the protein of the invention under conditions in which association of the protein with the binding partner

can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins. Alternatively, binding partners may be identified using a Far-Western assay according to the procedures of Takayama *et al., Methods Mol Biol* **69**:171-84, 1997 or Sauder *et al., J GenVirol* 77(5):991-6, 1996 or identified through the use of epitope tagged proteins or GST fusion proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

H. Methods to Identify Agents that Modulate the Expression a Nucleic Acid Encoding the Ischemic Heart Associated Protein.

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding a protein of the invention such as a protein having the amino acid sequence of SEQ ID No.2 or SEQ ID No.4. Such assays may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid of the invention, for instance a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4, if it is capable of up- or down-regulating expression of the nucleic acid in a cell.

In one assay format, cell lines that contain reporter gene fusions between the open reading frame defined by nucleotides 184-1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3 and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.* (1990) *Anal Biochem* **188**:245-254). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4.

Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding a protein of the invention such as the protein having SEQ ID No.2 or SEQ ID No.4. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press, 1989).

In order to assay gene expression of the present invention in a physiologically relevant manner, tissues may be analyzed under conditions which model physiological

cardiac cell stimuli. For example, some model systems simply include substrate depletion and increased intracellular acidity (Ch'en *et al., Prog Biophys Mol Biol* **69**(2-3):515-38, 1998). Others are more complex. For example, Wilders *et al.* used isolated guinea pig ventricular myocytes which were electrically coupled via a coupling-clamp circuit to a comprehensive computer model of a guinea pig ventricular myocyte to assess alterations in the critical value of coupling conductance required for action potential conduction from the real cells to the model cell when the real cells are exposed to a solution that simulates acute ischemia (Wilders *et al., Circulation* **30**;99(12):1623-9, 1999). Further, exposure of myocytes to tumor necrosis factor-alpha, IL-1beta, or lipopolysaccharide has been shown to simulate effects seen during reprofusion injury (Gwechenberger *et al., Circulation* **99**(4):546-51, 1999). Further, models have been developed to simulate ischemia and reprofusion in quiescent human ventricular cardiomyocytes. Cellular injury and metabolic parameters can be assessed after various interventions, such as: preconditioning cells with anoxia, hypoxia, anoxic supernatants, or hypoxic supernatants (Cohen *et al., Circulation* **98**(19 Suppl):II184-94; discussion II194-6, 1998). Another model is hypoxia-reoxygenation stress in the rat myoblast cell line, H9c2, which simulates ischemic preconditioning in heart tissue (Sakamoto *et al., Biochem Biophys Res Commun* **20**;251(2):576-9, 1998).

In a preferred embodiment, assays which incubate cells under conditions that simulate cardiac ischemia and/or heart stress *in vitro* include, but are not limited to, for example, fluid shear stress in human endothelial cells (Houston *et al., Artherioscler Thromb Vacs Biol* **19**(2):281-289, 1999) and passive stretch of cultured myocytes (Yamazaki *et al., J Mol Cel Cardiol* **27**(1):133-140, 1995). "Shear stress" refers to the energy necessary produce an opposite but parallel sliding motion across a body's plane. In a related aspect, "shear stress" refers to substantially the physiological equivalent pressure produced in various tissues or organs such as force present in the vasculature by the actions of cardiac muscle. In a related aspect, assays which simulate ischemia by stressing the heart *in vivo* include, but are not limited to, for example, occlusion of the heart by ligation of blood vessels in animal models (Soloman *et al., J Am Coll Cardiol* **33**(3): 854-856, 1999 and Kirma *et al., Jpn Circ J* **62**(4):294-298, 1998).

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, NY, 1989) or Ausubel *et al.* (Current Protocols in Molecular Biology, Greene Publishing Co., NY, 1995).

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.* and Ausubel *et al.* as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support and the solid support exposed to at least one probe comprising at least one, or part of one of the sequences of the invention under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a porous glass wafer. The glass wafer can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid encoding

the protein having the sequence of SEQ ID No.2 or SEQ ID No.4 are identified.

Hybridization for qualitative and quantitative analysis of mRNAs may also be carried out by using a RNase Protection Assay (*i.e.*, RPA, see Ma *et al.*, *Methods* **10**: 273-238, 1996). Briefly, an expression vehicle comprising cDNA encoding the gene product and a phage specific DNA dependent RNA polymerase promoter (e.g., T7, T3 or SP6 RNA polymerase) is linearized at the 3' end of the cDNA molecule, downstream from the phage promoter, wherein such a linearized molecule is subsequently used as a template for synthesis of a labeled antisense transcript of the cDNA by *in vitro* transcription. The labeled transcript is then hybridized to a mixture of isolated RNA (*i.e.*, total or fractionated mRNA) by incubation at 45°C overnight in a buffer comprising 80% formamide, 40 mM Pipes, pH 6.4, 0.4 M NaCl and 1 mM EDTA. The resulting hybrids are then digested in a buffer comprising 40 µg/ml ribonuclease A and 2 µg/ml ribonuclease. After deactivation and extraction of extraneous proteins, the samples are loaded onto urea/polyacrylamide gels for analysis.

In another assay format, agents which effect the expression of the instant gene products, cells or cell lines would first be identified which express said gene products physiologically (*e.g.*, see example Figure 1 for tissue distribution via Northern blot, however, RPAs may serve the identical purpose of expression selection). Cell and/or cell lines so identified would be expected to comprise the necessary cellular machinery such that the fidelity of modulation of the transcriptional apparatus is maintained with regard to exogenous contact of agent with appropriate surface transduction mechanisms and/or the cytosolic cascades. Further, such cells or cell lines would be transduced or transfected with an expression vehicle (*e.g.*, a plasmid or viral vector) construct comprising an operable non-translated 5'-promoter containing end of the structural gene encoding the instant gene products fused to one or more antigenic fragments, which are peculiar to the instant gene products, wherein said fragments are under the transcriptional control of said promoter and are expressed as polypeptides whose molecular weight can be distinguished from the naturally occurring polypeptides or may further comprise an immunologically distinct tag. Such a process is well known in the art (see Maniatis, 1982).

Cells or cell lines transduced or transfected as outlined above would then be contacted with agents under appropriate conditions; for example, the agent comprises a pharmaceutically acceptable excipient and is contacted with cells comprised in an aqueous physiological buffer such as phosphate buffered saline (PBS) at physiological pH, Eagles balanced salt solution (BSS) at physiological pH, PBS or BSS comprising serum or conditioned media comprising PBS or BSS and/or serum incubated at 37° C . Said conditions may be modulated as deemed necessary by one of skill in the art. Subsequent to contacting the cells with the agent, said cells will be disrupted and the polypeptides of the disruptate are fractionated such that a polypeptide fraction is pooled and contacted with an antibody to be further processed by immunological assay (e.g., ELISA, immunoprecipitation or Western blot). The pool of proteins isolated from the "agent contacted" sample will be compared with a control sample where only the excipient is contacted with the cells and an increase or decrease in the immunologically generated signal from the "agent contacted" sample compared to the control will be used to distinguish the effectiveness of the agent.

1. Methods to Identify Agents that Modulate at Least One Activity of the Ischemic Heart Associated Protein.

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of a protein of the invention such as the protein having the amino acid sequence of SEQ ID No.2 or SEQ ID No.4. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

In one format, the relative amounts of a protein of the invention between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptides. polypeptides or proteins of the invention if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the amino or carboxy terminus with a Cys residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein (*Nature* **256**(5517):495-7, 1975; *Eur J Immunol* **6**(7):511-9, 1976; and *Biotechnology* **24**:524-6, 1992)or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten. polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of $F(ab')_2$ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole

immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Antibody regions that bind specifically to the desired regions of the protein can also be produced in the context of chimeras with multiple species origin, for instance, humanized antibodies.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of the a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis which takes into account the sequence of the target site and/or its conformation in connection with the agent's action. As described in the Examples, there is a proposed tyrosine kinase phoshporylation site in the protein having SEQ ID No.2 or SEQ ID No.4. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up these sites. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to the putative tyrosine kinase phosphorylation site at amino acid 98 of SEQ ID No.1.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive with critical positions of proteins of the invention. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies.

J. Uses for Agents that Modulate at Least One Activity of the Ischemic Heart Associated Protein.

As provided in the Examples, the proteins and nucleic acids of the invention, such as the protein having the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, are expressed in ischemic heart tissue. Agents that modulate or down-regulate the expression of the protein or agents such as agonists or antagonists of at least one activity of the protein may be used to modulate biological and pathologic processes associated with the protein's function and activity.

As used herein, a subject can be any mammal, so long as the mammal is in need of modulation of a pathological or biological process mediated by a protein of the invention. The term "mammal" is meant an individual belonging to the class Mammalia. The invention is particularly useful in the treatment of human subjects.

Pathological processes refer to a category of biological processes which produce a deleterious effect. For example, expression of a protein of the invention may be associated with chronic ischemic heart disease and ischemic cardiomyopathy. As used herein, an agent is said to modulate a pathological process when the agent reduces the degree or severity of the process. For instance, chronic ischemic heart disease or ischemic cardiomyopathy may be prevented or disease progression modulated after an ischemic event by the administration of agents which reduce or modulate in some way the expression or at least one activity of a protein of the invention.

The agents of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. For example, an agent of the present invention can be administered in combination with anti-thrombotic agents. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a

fashion such that the agents will act at the same time.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The present invention further provides compositions containing one or more agents which modulate expression or at least one activity of a protein of the invention. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise 0.1 to 100 μg/kg body wt. The preferred dosages comprise 0.1 to 10 μg/kg body wt. The most preferred dosages comprise 0.1 to 1 μg/kg body wt.

In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin

capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions according to generally accepted medical practice, such as anticoagulant agents, thrombolytic agents, or other antithrombotics, including platelet aggregation inhibitors, tissue plasminogen activators, urokinase, prourokinase, streptokinase, heparin, aspirin, or warfarin. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

K. Transgenic Animals

Transgenic animals containing mutant, knock-out or modified genes corresponding to the cDNA sequence of SEQ ID NO:1 or SEQ ID NO: 3 are also included in the invention. Transgenic animals are genetically modified animals into which recombinant, exogenous or cloned genetic material has been experimentally transferred. Such genetic material is often referred to as a "transgene". The nucleic acid sequence of the transgene, in this case a form of SEQ ID NO: 1 or SEQ ID NO: 3, may be integrated either at a locus of a genome where that particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal.

The term "germ cell line transgenic animal" refers to a transgenic animal in which the genetic alteration or genetic information was introduced into a germ line cell, thereby conferring the ability of the transgenic animal to transfer the genetic information to offspring. If such offspring in fact possess some or all of that alteration or genetic information, then they too are transgenic animals.

The alteration or genetic information may be foreign to the species of animal to

which the recipient belongs, foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

Transgenic animals can be produced by a variety of different methods including transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection (*see, e.g.*, U.S. Patent No. 4,736,866; U.S. Patent No. 5,602,307; Mullins *et al.*, *Hypertension* **22**(4):630-633, 1993; Brenin *et al.*, *Surg Oncol* **6**(2)99-110, 1997; Tuan (ed.), Recombinant Gene Expression Protocols, Methods in Molecular Biology, 1997, No. 62, Humana Press).

A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express simian SV 40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.*, *Genetics* **143**(4):1753-1760, 1996; or, are capable of generating a fully human antibody response (McCarthy, *The Lancet* **349**(9049):405, 1997).

While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, pigs, dogs, cats, monkeys, chimpanzees, hamsters, rabbits, cows and guinea pigs (*see, e.g.*, Kim *et al.*, *Mol Reprod Dev* **46**(4):515-526, 1997; Houdebine, *Reprod Nutr Dev* **35**(6):609-617, 1995; Petters *Reprod Fertil Dev* **6**(5):643-645, 1994 ; Schnieke *et al.*, *Science* **278**(5346):2130-2133, 1997; and Amoah *J Animal Science* **75**(2):578-585, 1997).

The method of introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method which favors co-transformation of

multiple nucleic acid molecules. Detailed procedures for producing transgenic animals are readily available to one skilled in the art, including the disclosures in U.S. Patent No. 5,489,743 and U.S. Patent No. 5,602,307.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1

Identification of Differentially Expressed Ischemic Heart mRNA

Heart tissue was obtained from five male patients with inotrope-dependent post-ischemic cardiomyopathy exhibiting severe myocyte and or cardiac hypertrophy with at least three years since their first myocardial infarction. Heart tissue was also obtained from 5 female patients with idiopathic dilated cardiomyopathy exhibiting severe myocyte and/or cardiac hypertrophy and CHF duration of at least 2 years.

Total cellular RNA was prepared from the heart tissue described above as well as from control, non-ischemic heart tissue using the procedure of Newburger *et al.*, *J. Biol. Chem.* **266**(24):16171-7, 1981 and Newburger *et al.*, *Proc Natl Acad Sci USA* **85**:5215-5219, 1988.

Synthesis of cDNA was performed as previously described by Prashar *et al.* in WO 97/05286 and in Prashar *et al., Proc Natl Acad Sci. USA* **93**:659-663, 1996. Briefly, cDNA was synthesized according to the protocol described in the GIBCO/BRL kit for cDNA synthesis. The reaction mixture for first-strand synthesis included 6 μg of total RNA, and 200 ng of a mixture of 1-base anchored oligo(dT) primers with all three possible anchored bases (ACGTAATACGACTCACTATAGGGCGAATTGGGTCGACTTTTTTTTTTTTTTT Tn1 wherein n1=A/C or G) (SEQ ID NO:5) along with other components for first-strand synthesis reaction except reverse transcriptase. This mixture was incubated at 65°C for 5m, chilled on ice and the process repeated. Alternatively, the reaction mixture may include 10μg of total RNA, and 2 pmol of 1 of the 2-base anchored oligo(dT) primers a heel such as RP5.0 (CTCTCAAGGATCTTACCGCTT $_{18}$AT) (SEQ ID NO:6), or RP6.0 (TAATACCGCGCCACATAGCAT $_{18}$CG) (SEQ ID NO:7), or RP9.2 (CAGGGTAGACGACGCTACGCT$_{18}$GA) (SEQ ID NO: 8) along with other components for first-strand synthesis reaction except reverse transcriptase. This mixture

was then layered with mineral oil and incubated at 65°C for 7 min followed by 50°C for another 7 min. At this stage, 2μl of Superscript reverse transcriptase (200 units/μl; GIBCO/BRL) was added quickly and mixed, and the reaction continued for 1 hr at 45-50°C. Second-strand synthesis was performed at 16°C for 2 hr. At the end of the reaction, the cDNAs were precipitated with ethanol and the yield of cDNA was calculated. In our experiments, ≈200 ng of cDNA was obtained from 10μg of total RNA.

The adapter oligonucleotide sequences were

A1 (TAGCGTCCGGCGCAGCGACGGCCAG) (SEQ ID NO:9)and

A2 (GATCCTGGCCGTCGGCTGTCTGTCGGCGC) (SEQ ID NO:10). One microgram of oligonucleotide A2 was first phosphorylated at the 5′ end using T4 polynucleotide kinase (PNK). After phosphorylation, PNK was heated denatured, and 1μg of the oligonucleotide A1 was added along with 10× annealing buffer (1 M NaC1/100 mM Tris-HCl, pH8.0/10 mM EDTA, pH8.0) in a final vol of 20 μl. This mixture was then heated at 65°C for 10 min followed by slow cooling to room temperature for 30 min, resulting in formation of the Y adapter at a final concentration of 100 ng/μl. About 20 ng of the cDNA was digested with 4 units of *Bgl* II in a final vol of 10 μl for 30 min at 37°C. Two microliters (≈4 ng of digested cDNA) of this reaction mixture was then used for ligation to 100 ng (≈50-fold) of the Y-shaped adapter in a final vol of 5μl for 16 hr at 15°C. After ligation, the reaction mixture was diluted with water to a final vol of 80 μl (adapter ligated cDNA concentration, ≈50 pg/μl) and heated at 65°C for 10 min to denature T4 DNA ligase, and 2-μl aliquots (with ≈100 pg of cDNA) were used for PCR.

The following sets of primers were used for PCR amplification of the adapter ligated 3′ -end cDNAs:

TGAAGCCGAGACGTCGGTCG$(T)_{18}$ n1, n2 (SEQ ID NO:11) (wherein n1, n2 = AA, AC, AG AT CA CC CG CT GA GC GG and GT) as the 3' primer with A1 as the 5' primer or alternatively

RP 5.0, RP 6.0, or RP 9.2 used as 3′ primers with primer A1.1 serving as the 5′ primer. To detect the PCR products on the display gel, 24 pmol of oligonucleotide A1

or A1.1 was 5′ -end-labeled using 15 μl of [γ-^{32} P]ATP (Amersham; 3000 Ci/mmol) and PNK in a final volume of 20 μl for 30 min at 37°C. After heat denaturing PNK at 65°C for 20 min, the labeled oligonucleotide was diluted to a final concentration of 2 μM in 80 μl with unlabeled oligonucleotide A1.1. The PCR mixture (20μl) consisted of 2 μl (≈100 pg) of the template, 2μl of 10× PCR buffer (100 mM Tris·HCl, pH 8.3/500 mM KCl), 2 μl of 15 mM $MgCl_2$ to yield 1.5 mM final Mg^{2+} concentration optimum in the reaction mixture, 200 μM dNTPs, 200 nM each 5′ and 3′ PCR primers, and 1 unit of Amplitaq Gold®. Primers and dNTPs were added after preheating the reaction mixture containing the rest of the components at 85°C. This "hot start" PCR was done to avoid artefactual amplification arising out of arbitrary annealing of PCR primers at lower temperature during transition from room temperature to 94°C in the first PCR cycle. PCR consisted of 5 cycles of 94°C for 30 sec, 55°C for 2 min, and 72°C for 60 sec followed by 25 cycles of 94°C for 30 sec, 60°C for 2 min, and 72°C for 60 sec. A higher number of cycles resulted in smeary gel patterns. PCR products (2.5μl) were analyzed on 6% polyacrylamide sequencing gel. For double or multiple digestion following adapter ligation, 13.2 μl of the ligated cDNA sample was digested with a secondary restriction enzyme(s) in a final vol of 20 μl. From this solution, 3μl was used as template for PCR. This template vol of 3 μl carried ≈ 100 pg of the cDNA and 10 mM $MgCl_2$ (from the 10× enzyme buffer), which diluted to the optimum of 1.5 mM in the final PCR vol of 20 μl. Since Mg^{2+} comes from the restriction enzyme buffer, it was not included in the reaction mixture when amplifying secondarily cut cDNA. Individual cDNA fragments corresponding to mRNA species were separated by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography. Bands were extracted from the display gels as described by Liang *et al.* (*Cur Opin Immunol* 7:274-280, 1995), reamplified using the 5′ and 3′ primers, and subcloned into pCR-Script with high efficiency using the PCR-Script cloning kit from Stratagene. Plasmids were sequenced by cycle sequencing on an ABI automated sequencer. Alternatively, bands were extracted (cored) from the display gels, PCR amplified and sequenced directly without subcloning.

Fragment 3145 is a band that corresponds to a cDNA derived from a mRNA species that is expressed in at least one ischemic heart tissue sample. The band corresponding to fragment 3145 was sequenced. The sequence of the band is: ccaggagctatgaatgactcagtggtggaaatgcccttctggaaactgaatattaccttctgtaggaaaaggtggaaaataagc atctagaaggttgttgtgaatgactctgtgctggcaaaaatgcttgaaacctctatatttctttcgttcataagacgtaaaggtcaaat ttttcaagaaaagtcttttaataacaaaagcatgcagttctctgtgaaatctcaaatattgttgtaatagtctgtttcaatcttaaaaaga atcaataaaaacaaacaagggaaaa (SEQ ID NO:12).

Example 2

Cloning of a Full Length cDNA Corresponding to 3145

The full length cDNA corresponding to band 3145 was obtained by the oligo-pulling method. Briefly, a gene-specific oligo was designed based on cDNA fragment 3145. The oligo was labeled with biotin and used to hybridize with 2 ug of single strand plasmid DNA (cDNA recombinants) from a human heart cDNA library following the procedures of Sambrook *et al.*. The hybridized cDNAs were separated by streptavidin-conjugated beads and eluted by heating. The eluted cDNA was converted to double strand plasmid DNA and used to transform *E. coli* cells (DH10B) and the longest cDNA was screened. After confirmed by PCR using gene-specific primers, the cDNA clone was subjected to DNA sequencing.

The nucleotide sequence of the full-length cDNA corresponding to the differentially regulated band 3145 band is set forth in SEQ ID No:1 (PGP-980.1). The cDNA comprises 2008 base pairs with an open reading frame encoding a protein predicted to contain 339 amino acids. The predicted amino acid sequence is presented in SEQ ID Nos. 1 and 2.

The nucleotide sequence of a second full length cDNA corresponding to the differentially regulated band 3145 is set forth in SEQ ID NO:3 (PGP980.2). The cDNA comprises 1981 base pairs with an open reading frame encoding a protein predicted to contain 339 amino acids. The predicted amino acid sequence is set forth in SEQ ID

Nos: 3 and 4. The 339 amino acid sequence of PGP980.1 and PGP980.2 differ only by the presence of a cysteine residue at amino acid residue 66 in PGP980.1 and an arginine at the same amino acid residue in PGP980.2.

The predicted isoelectric point of the 2 proteins is approximately 7.52. Both proteins contain a putative signal sequence comprising amino acids 1-29.

Example 3

Northern blot and PCR Expression Analysis

The tissue distribution of RNA encoding the differentially regulated gene encoding the protein of SEQ ID NO:2 was analyzed by Northern blot as well as PCR expression analysis of RNA isolated from various tissues. RNA was isolated from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas using standard procedures. Northern blots were prepared using a probe derived from SEQ ID NO:1 with hybridization conditions as described by Sambrook *et al* (1989). PCR expression analysis was also performed using primers derived from SEQ ID NO:1 using AmpliTaq Gold PCR® amplification kits (Perkin Elmer). Figure 1 is a Northern blot demonstrating the presence of variable levels of specific RNA in all tissues.

Quantitative PCR Analysis of Expression Levels

Figure 2 is a PCR analysis of expression levels in normal and ischemic heart tissue samples compared to the detected levels in the differential display. Samples 206 and 558 are normal heart tissue samples. Samples 146, 149, 294, 320 and 327 are ischemic heart tissue samples (see Table 1). The first fragment in the top row of Fgure 2 is a differential display band from a normal heart tissue samples. Bands 2-6 correspond to the differential display bands for samples 146, 149, 294, 320 and 327, respectively.

Real time PCR detection was accomplished by the use of the ABI PRISM 7700 Sequence Detection System. The 7700 measures the fluorescence intensity of the sample each cycle and is able to detect the presence of specific amplicons within the PCR reaction. Each sample was assayed for the level of GAPDH and Clone 980.

GAPDH detection was performed using Perkin Elmer part#402869 according to the manufacturer's directions. Primers were designed for clone 980 using Primer Express, a program developed by PE to efficiently find primers and probes for specific sequences. These primers were used in conjunction with SYBR green (Molecular Probes), a nonspecific double stranded DNA dye, to measure the expression level of a clone 980, which was normalized to the GAPDH level in each sample. The normalized values are reported in Figure 2. Samples 206 and 558 are normal samples, the remainder are from ischemic male patients.

GL Sample	File #	Age	Gender	Pt Wt	Pt Ht	Heart Wt	Etiology
S00146A	20	66	Male	70	175	600	ischemic
S00149A	26	65	Male	94	178	724	ischemic
S00294A	73	66	Male	86	169	519	ischemic
S00320A	109	60	Male	82	168	562	ischemic
S00327A	116	67	Male	71	183	619	ischemic

Example 4

Method of Screening for Modulators of PGP980.1 and PGP980.2 Expression Using Shear-Stress Assay

Using human epithelial cells, a shear-stress of 1.5 N/m^2 is applied to cells in culture according to the method of Houston *et al.* (*Artherioscler Thromb Vacs Biol* 19(2):281-289, 1999). At specific time points during applied stress, candidate agents and diluent (*i.e.*, carrier minus agent; control) are contacted with human epithelial cells. Cells are removed and lysed in an appropriate buffer for isolation of total and/or messenger RNA in a similar fashion as described in Sambrook *et al.* (1989). Isolated nucleic acids are then assayed by a transcriptional profiling means to determine whether the candidate agent modulates the induction of PGP980.1 and PGP980.2. Agents which up- or down-regulate the expression of either one or both transcripts are then designated as modulators of PGP980.1 and PGP980.2.

Example 5

Method of Screening for Modulators of Myocardial PGP980.1 and PGP980.2 Expression Using Passive Stretch of Cardiomyocytes

Using cultured myocytes on silicone membranes, cells are passively stretched according to the method of Yamazaki *et al.* (*J Mol Cell Cardiol* 27(1):133-140, 1995). At specific time points during applied stress, candidate agents and diluent (*i.e.*, carrier minus agent; control) are contacted with human epithelial cells. Control and test cells are removed and lysed in an appropriate buffer for isolation of total and/or messenger RNA in a similar fashion as described in Sambrook *et al* (1989). Isolated nucleic acids are then assayed by a transcriptional profiling assay to determine whether the candidate agent modulates the induction of PGP980.1 and PGP980.2. Agents which up- or down-regulate the expression of either one or both transcripts will then be designated as modulators of PGP980.1 and PGP980.2.

Example 6

Method of Screening for Modulators of Myocardial PGP980.1 and PGP980.2

Expression Using an Animal Model for Occlusion and Reprofusion of the Heart

Animal models for occlusion of the heart are well documented (Soloman *et al., J Am Coll Cardiol* 33(3): 854-856, 1999 and Kirma *et al., Jpn Circ J* 62(4):294-298, 1998). For example, pigs are used wherein regional ischemia is produced in control and candidate agent treated animals by partially occluding (ligating) the left anterior descending coronary artery. After obtaining the baseline values for evaluation of heart rate, transmitral flow and blood pressure, agents are administered to the animals (including carrier-only for controls) and at various time points and/or after administration of various concentrations of candidate agents using a single time point, post occlusion, the hearts of the animals are removed for isolation of nucleic acids by standard methods as described in Sambrook *et al* (1989). Isolated nucleic acids are then assayed by a transcriptional profiling assay to determine whether the candidate agent modulates the induction of PGP980.1 and PGP980.2. Agents which up- or down-regulate of either one or both transcripts will then be designated as modulators of PGP980.1 and PGP980.2.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents and publications referred to in this application are herein incorporated by reference in their entirety.

WHAT IS CLAIMED:

1. An isolated nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4; (b) an isolated nucleic acid molecule that encodes a fragment of at least 6 amino acids of SEQ ID No.2 or SEQ ID No.4; (c) an isolated nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule comprising SEQ ID No.1 or SEQ ID No.3 under conditions of sufficient stringency to produce a clear signal; and (d) an isolated nucleic acid molecule which hybridizes to a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No.2 or SEQ ID No.4 under conditions of sufficient stringency to produce a clear signal.

2. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises the sequence of SEQ ID No.1 or SEQ ID No.3.

3. The isolated nucleic acid molecule of claim 2, wherein the nucleic acid molecule consists of the sequence of SEQ ID No.1 or SEQ ID No.3.

4. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises nucleotides 184 to 1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleic acid molecule consists of nucleotides 184 to 1200 of SEQ ID No.1 or nucleotides 133-1149 of SEQ ID No.3.

6. The isolated nucleic acid molecule of any one of claims 1-5, wherein said nucleic acid molecule is operably linked to one or more expression control elements.

7. A vector comprising an isolated nucleic acid molecule of any one of claims 1-5.

8. A host cell transformed to contain the nucleic acid molecule of any one claims 1-5.

9. A host cell comprising a vector of claim 7.

10. A host cell of claim 9, wherein said host is selected from the group consisting of prokaryotic hosts and eukaryotic hosts.

11. A method for producing a polypeptide comprising the step of culturing a host cell transformed with the nucleic acid molecule of any one of claims 1-5 under conditions in which the protein encoded by said nucleic acid molecule is expressed.

12. The method of claim 11, wherein said host cell is selected from the group consisting of prokaryotic hosts and eukaryotic hosts.

13. An isolated polypeptide produced by the method of claim 11.

14. An isolated polypeptide selected from the group consisting of an isolated polypeptide comprising the amino acid sequence of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising a fragment of at least 6 amino acids of SEQ ID No.2 or SEQ ID No.4, an isolated polypeptide comprising conservative amino acid substitutions of SEQ ID No.2 or SEQ ID No.4 and naturally occurring amino acid sequence variants of SEQ ID No.2 or SEQ ID No.4.

15. An isolated antibody that binds to a polypeptide of either claim 13 or 14.

16. The antibody of claim 14 wherein said antibody is a monoclonal or polyclonal antibody.

17. A method of identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the steps of:

exposing cells which express the nucleic acid to the agent; and

determining whether the agent modulates expression of said nucleic acid, thereby identifying an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4.

18. A method of identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the steps of:

exposing cells which express the protein to the agent;

determining whether the agent modulates at least one activity of said protein, thereby identifying an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

19. The method of claim 19, wherein the agent modulates the phosphorylation of the protein.

20. A method of identifying binding partners for a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4, comprising the steps of:

exposing said protein to a potential binding partner; and

determining if the potential binding partner binds to said protein, thereby identifying binding partners for a protein comprising the sequence of SEQ ID No 2 or SEQ ID No.4.

21. A method of modulating the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the step of:

administering an effective amount of an agent which modulates the expression of a nucleic acid encoding the protein having the sequence of SEQ ID No.2 or SEQ ID

No.4.

22. A method of modulating at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4 comprising the step of:

administering an effective amount of an agent which modulates at least one activity of a protein comprising the sequence of SEQ ID No.2 or SEQ ID No.4.

23. A method of screening for agents which modulate PGP980.1 gene expression comprising the steps:

a) incubating cells under conditions which simulate heart stress or cardiac ischemia in the presence or absence of an agent;

b) determining whether PGP980.1 is up- or down-regulated in the presence of the agent, wherein up- or down-regulation of PGP980.1 identifies the agent as a modulator.

24. A method of screening for agents which modulate PGP980.2. gene expression comprising the steps:

a) incubating cells under conditions which simulate heart stress or cardiac ischemia in the presence or absence of an agent;

b) determining whether PGP980.2. is up- or down regulated in the presence of the agent, wherein up- or down-regulation of PGP980.2. induction identifies the agent as a modulator.

25. The method of claim 23, wherein the incubation conditions comprise shear stress.

26. The method of claim 23, wherein the incubation conditions comprise passive stretch of myocytes.

27. The method of claim 24, wherein the incubation conditions comprise shear stress.

28. The method of claim 24, wherein the incubation conditions comprise passive stretch of myocytes.

29. A method of screening for agents which modulate PGP980.1 gene expression comprising the steps:

a) occluding blood vessels leading to the heart of test and control animals to produce conditions which simulate cardiac ischemia and

b) determining whether PGP980.1 is up-or down-regulated in the presence of an administered agent, wherein up or down regulation of PGP980.1 identifies the agent as a modulator.

30. A method of screening for agents which modulate PGP980.2. gene expression comprising the steps:

a) occluding blood vessels leading to the heart of test and control animals to produce conditions which simulate cardiac ischemia and

b) determining whether PGP980.2. is up-or down-regulated in the presence of an administered agent, wherein up-or down-regulation of PGP980.2. identifies the agent as a modulator.

31. A non-human transgenic animal comprising a nucleic acid molecule of any of claims 1-6.

32. A non-human transgenic animal which does not express a nucleic acid molecule of any of claims 1-6.

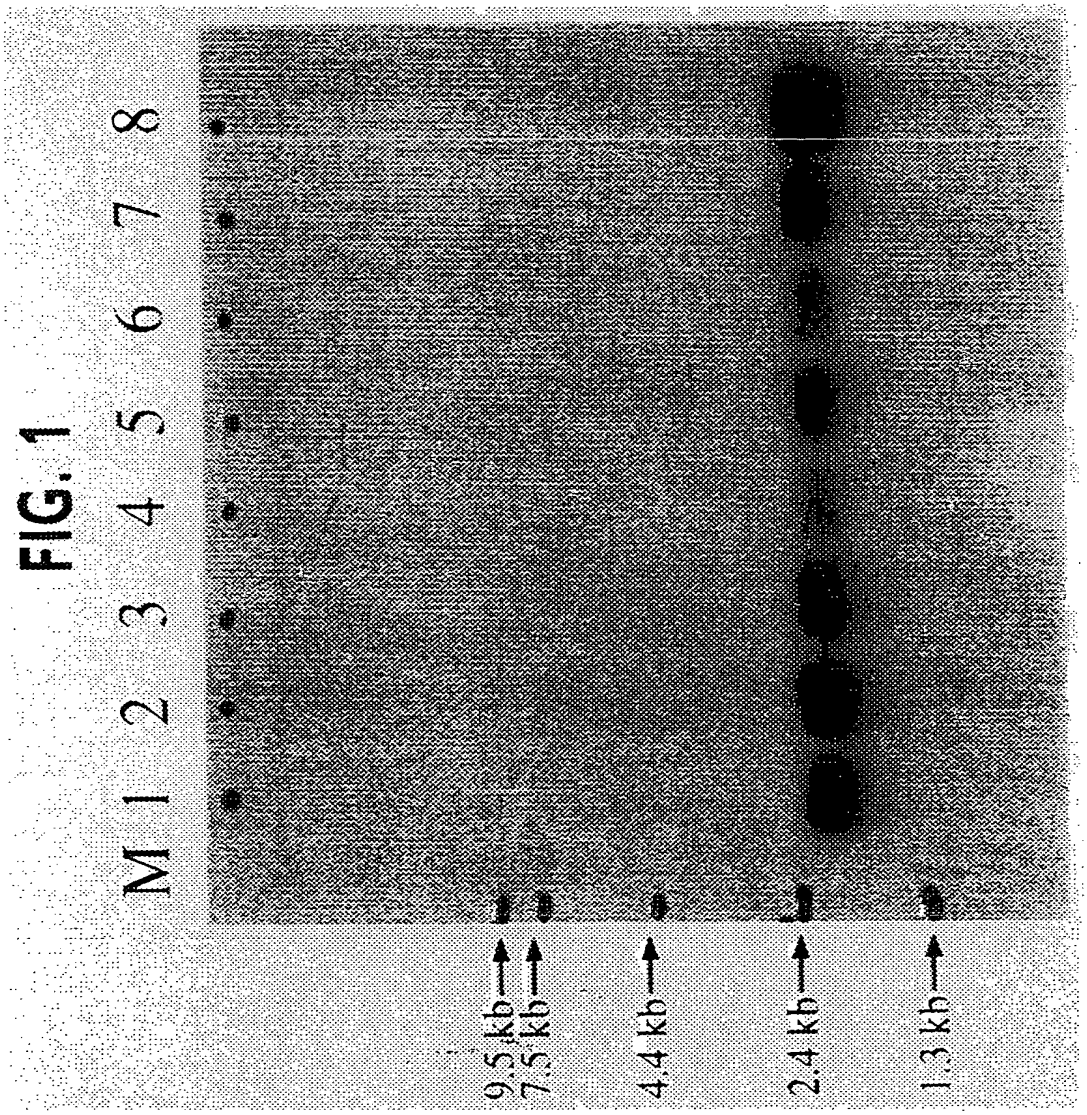
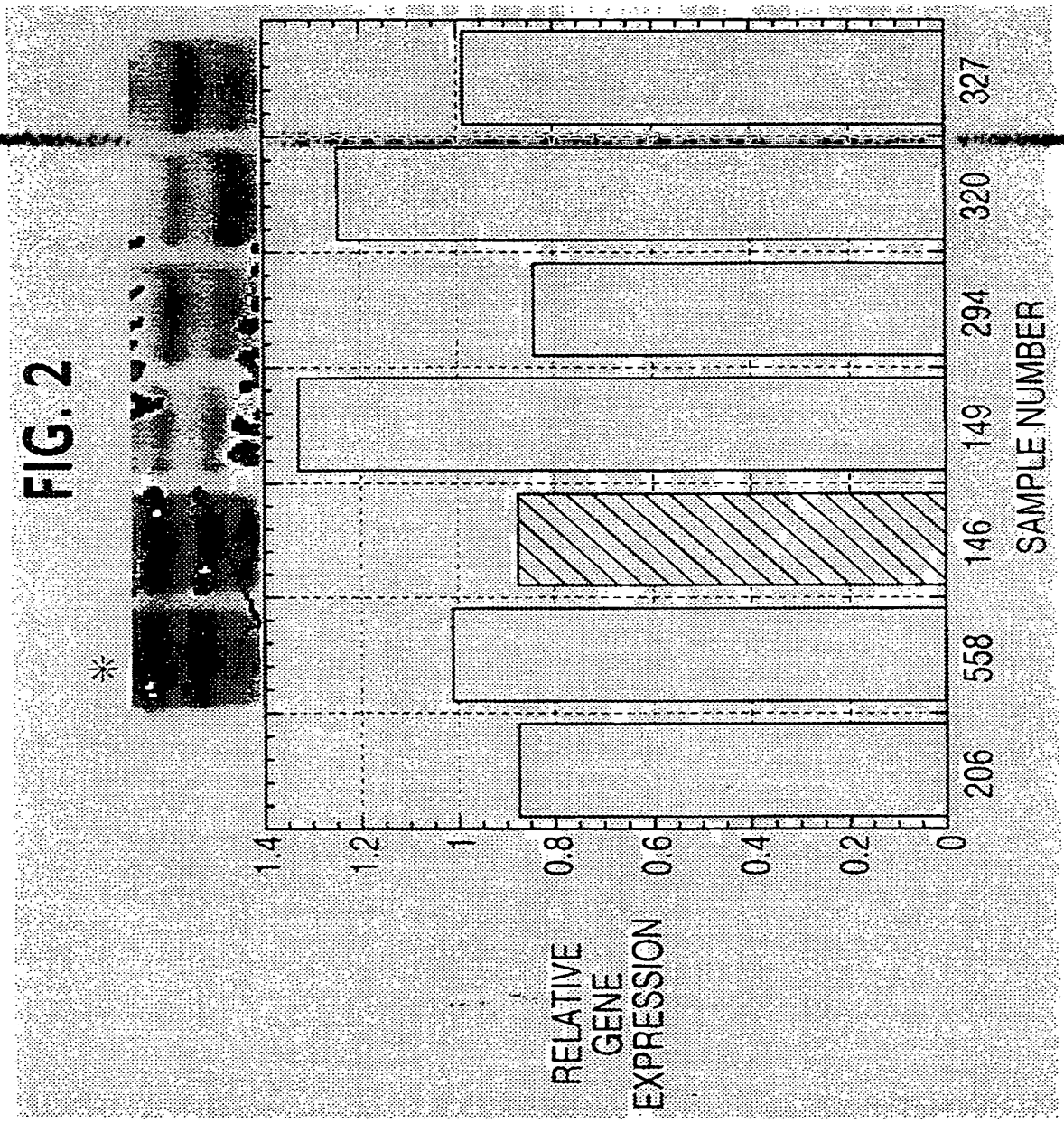


FIG. 1

FIG. 2



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Einstein, Richard

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1 5 10 15

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Leu Gly Leu His Leu Phe Leu Leu Thr Ala Gly Pro Ala Leu Gly Trp
20 25 30

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Asn Asp Pro Asp Arg Met Leu Leu Arg Asp Val Lys Ala Leu Thr Leu
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Leu Lys Cys Val Gly Gly Thr Ala Gly Cys Asp Ser Tyr Thr Pro Lys
      65                  70                  75

gtc ata cag tgt cag aac aaa ggc tgg gat ggg tat gat gta cag tgg   468
Val Ile Gln Cys Gln Asn Lys Gly Trp Asp Gly Tyr Asp Val Gln Trp
 80                  85                  90                  95

gaa tgt aag acg gac tta gat att gca tac aaa ttt gga aaa act gtg   516
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                100                 105                 110

gtg agc tgt gaa ggc tat gag tcc tct gaa gac cag tat gta cta aga   564
Val Ser Cys Glu Gly Tyr Glu Ser Ser Glu Asp Gln Tyr Val Leu Arg
            115                 120                 125

ggt tct tgt ggc ttg gag tat aat tta gat tat aca gaa ctt ggc ctg   612
Gly Ser Cys Gly Leu Glu Tyr Asn Leu Asp Tyr Thr Glu Leu Gly Leu
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cag aaa ctg aag gag tct gga aag cag cac ggc ttt gcc tct ttc tct   660
Gln Lys Leu Lys Glu Ser Gly Lys Gln His Gly Phe Ala Ser Phe Ser
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gat tat tat tat aag tgg tcc tcg gcg gat tcc tgt aac atg agt gga   708
Asp Tyr Tyr Tyr Lys Trp Ser Ser Ala Asp Ser Cys Asn Met Ser Gly
160                 165                 170                 175

ttg att acc atc gtg gta ctc ctt ggg atc gcc ttt gta gtc tat aag   756
Leu Ile Thr Ile Val Val Leu Leu Gly Ile Ala Phe Val Val Tyr Lys
                180                 185                 190

ctg ttc ctg agt gac ggg cag tat tct cct cca ccg tac tct gag tat   804
Leu Phe Leu Ser Asp Gly Gln Tyr Ser Pro Pro Pro Tyr Ser Glu Tyr
            195                 200                 205

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Pro Pro Phe Ser His Arg Tyr Gln Arg Phe Thr Asn Ser Ala Gly Pro
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cct ccc cca ggc ttt aag tct gag ttc aca gga cca cag aat act ggc   900
Pro Pro Pro Gly Phe Lys Ser Glu Phe Thr Gly Pro Gln Asn Thr Gly
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tat gaa aat tca gga cca ggg ttc tgg aca ggc ttg gga act ggt gga   996
Tyr Glu Asn Ser Gly Pro Gly Phe Trp Thr Gly Leu Gly Thr Gly Gly
                260                 265                 270

ata cta gga tat ttg ttt ggc agc aat aga gcg gca aca ccc ttc tca  1044
Ile Leu Gly Tyr Leu Phe Gly Ser Asn Arg Ala Ala Thr Pro Phe Ser
            275                 280                 285

gac tcg tgg tac tac ccg tcc tat cct ccc tcc tac cct ggc acg tgg  1092
Asp Ser Trp Tyr Tyr Pro Ser Tyr Pro Pro Ser Tyr Pro Gly Thr Trp
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aat agg gct tac tca ccc ctt cat gga ggc tcg ggc agc tat tcg gta  1140
Asn Arg Ala Tyr Ser Pro Leu His Gly Gly Ser Gly Ser Tyr Ser Val
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tgt tca aac tca gac acg aaa acc aga act gca tca gga tat ggt ggt  1188
Cys Ser Asn Ser Asp Thr Lys Thr Arg Thr Ala Ser Gly Tyr Gly Gly
320                 325                 330                 335

acc agg aga cga taaagtagaa agttggagtc aaacactgga tgcagaaatt      1240
Thr Arg Arg Arg

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Asp Pro Asp Arg Met Leu Leu Arg Asp Val Lys Ala Leu Thr Leu His
         35                  40                  45

Tyr Asp Arg Tyr Thr Thr Ser Arg Arg Leu Asp Pro Ile Pro Gln Leu
     50                  55                  60

Lys Cys Val Gly Gly Thr Ala Gly Cys Asp Ser Tyr Thr Pro Lys Val
 65                  70                  75                  80

Ile Gln Cys Gln Asn Lys Gly Trp Asp Gly Tyr Asp Val Gln Trp Glu
                 85                  90                  95

Cys Lys Thr Asp Leu Asp Ile Ala Tyr Lys Phe Gly Lys Thr Val Val
            100                 105                 110

Ser Cys Glu Gly Tyr Glu Ser Ser Glu Asp Gln Tyr Val Leu Arg Gly
        115                 120                 125

Ser Cys Gly Leu Glu Tyr Asn Leu Asp Tyr Thr Glu Leu Gly Leu Gln
    130                 135                 140

Lys Leu Lys Glu Ser Gly Lys Gln His Gly Phe Ala Ser Phe Ser Asp
145                 150                 155                 160

Tyr Tyr Tyr Lys Trp Ser Ser Ala Asp Ser Cys Asn Met Ser Gly Leu
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Ile Thr Ile Val Val Leu Leu Gly Ile Ala Phe Val Val Tyr Lys Leu
            180                 185                 190
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Pro Pro Gly Phe Lys Ser Glu Phe Thr Gly Pro Gln Asn Thr Gly His
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Gly Ala Thr Ser Gly Phe Gly Ser Ala Phe Thr Gly Gln Gln Gly Tyr
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Glu Asn Ser Gly Pro Gly Phe Trp Thr Gly Leu Gly Thr Gly Gly Ile
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Leu Gly Tyr Leu Phe Gly Ser Asn Arg Ala Ala Thr Pro Phe Ser Asp
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tcatcgagcg cc atg gcc gca gcc tgc ggg ccg gga gcg gcc ggg tac tgc 171
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ggc tgg aac gac cct gac aga atg ttg ctg cgg gat gta aaa gct ctt   267
Gly Trp Asn Asp Pro Asp Arg Met Leu Leu Arg Asp Val Lys Ala Leu
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acc ctc cac tat gac cgc tat acc acc tcc cgc agg ctg gat ccc atc   315
Thr Leu His Tyr Asp Arg Tyr Thr Thr Ser Arg Arg Leu Asp Pro Ile
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cca cag ttg aaa cgt gtt gga ggc aca gct ggt tgt gat tct tat acc   363
Pro Gln Leu Lys Arg Val Gly Gly Thr Ala Gly Cys Asp Ser Tyr Thr
             65                  70                  75

cca aaa gtc ata cag tgt cag aac aaa ggc tgg gat ggg tat gat gta   411
Pro Lys Val Ile Gln Cys Gln Asn Lys Gly Trp Asp Gly Tyr Asp Val
         80                  85                  90

cag tgg gaa tgt aag acg gac tta gat att gca tac aaa ttt gga aaa   459
Gln Trp Glu Cys Lys Thr Asp Leu Asp Ile Ala Tyr Lys Phe Gly Lys
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act gtg gtg agc tgt gaa ggc tat gag tcc tct gaa gac cag tat gta   507
Thr Val Val Ser Cys Glu Gly Tyr Glu Ser Ser Glu Asp Gln Tyr Val
110                 115                 120                 125

cta aga ggt tct tgt ggc ttg gag tat aat tta gat tat aca gaa ctt   555
Leu Arg Gly Ser Cys Gly Leu Glu Tyr Asn Leu Asp Tyr Thr Glu Leu
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ggc ctg cag aaa ctg aag gag tct gga aag cag cac ggc ttt gcc tct   603
Gly Leu Gln Lys Leu Lys Glu Ser Gly Lys Gln His Gly Phe Ala Ser
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Ser Gly Leu Ile Thr Ile Val Val Leu Leu Gly Ile Ala Phe Val Val
    175                 180                 185

tat aag ctg ttc ctg agt gac ggg cag tat tct cct cca ccg tac tct   747
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gga cct cct ccc cca ggc ttt aag tct gag ttc aca gga cca cag aat   843
Gly Pro Pro Pro Pro Gly Phe Lys Ser Glu Phe Thr Gly Pro Gln Asn
            225                 230                 235

act ggc cat ggt gca act tct ggt ttt ggc agt gct ttt aca gga caa   891
Thr Gly His Gly Ala Thr Ser Gly Phe Gly Ser Ala Phe Thr Gly Gln
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caa gga tat gaa aat tca gga cca ggg ttc tgg aca ggc ttg gga act   939
Gln Gly Tyr Glu Asn Ser Gly Pro Gly Phe Trp Thr Gly Leu Gly Thr
    255                 260                 265

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Thr Trp Asn Arg Ala Tyr Ser Pro Leu His Gly Gly Ser Gly Ser Tyr
            305                 310                 315

tcg gta tgt tca aac tca gac acg aaa acc aga act gca tca gga tat   1131
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        320                 325                 330

ggt ggt acc agg aga cga taaagtagaa agttggagtc aaacactgga         1179
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         35                  40                  45

Tyr Asp Arg Tyr Thr Thr Ser Arg Arg Leu Asp Pro Ile Pro Gln Leu
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Lys Arg Val Gly Gly Thr Ala Gly Cys Asp Ser Tyr Thr Pro Lys Val
 65                  70                  75                  80

Ile Gln Cys Gln Asn Lys Gly Trp Asp Gly Tyr Asp Val Gln Trp Glu
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Cys Lys Thr Asp Leu Asp Ile Ala Tyr Lys Phe Gly Lys Thr Val Val
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Ser Cys Glu Gly Tyr Glu Ser Ser Glu Asp Gln Tyr Val Leu Arg Gly
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Tyr Tyr Tyr Lys Trp Ser Ser Ala Asp Ser Cys Asn Met Ser Gly Leu
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Phe Leu Ser Asp Gly Gln Tyr Ser Pro Pro Pro Tyr Ser Glu Tyr Pro
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Pro Phe Ser His Arg Tyr Gln Arg Phe Thr Asn Ser Ala Gly Pro Pro
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Pro Pro Gly Phe Lys Ser Glu Phe Thr Gly Pro Gln Asn Thr Gly His
225                 230                 235                 240

Gly Ala Thr Ser Gly Phe Gly Ser Ala Phe Thr Gly Gln Gln Gly Tyr
                245                 250                 255

Glu Asn Ser Gly Pro Gly Phe Trp Thr Gly Leu Gly Thr Gly Gly Ile
            260                 265                 270

Leu Gly Tyr Leu Phe Gly Ser Asn Arg Ala Ala Thr Pro Phe Ser Asp
        275                 280                 285

Ser Trp Tyr Tyr Pro Ser Tyr Pro Pro Ser Tyr Pro Gly Thr Trp Asn
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product of PGP 980 gene

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20015

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/8; 514/1; 536/23.5; 435/320.1, 325, 252.1, 326, 6, 7.1, 375; 530/350, 387.1,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, CAPLUS, WEST 1.1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank, Accession No. AA001371, HILLIER L et al. 'The WashU-Merck Project', abstract, unpublished, 29 November 1996. See sequence comparison.	1
X, P --- Y, P	WO 98/46757 A2 (GENETICS INSTITUTE, INC.) 22 October 1998, see entire document. Also see sequence comparison with N_Geneseq Accession No v62752.	1-14 --- 15-32
X, P --- Y, P	WO 98/54206 A1 (HUMAN GENOME SCIENCES, INC.) 03 December 1998, see entire document. Also see sequence comparison with N_Geneseq Accession No V08330.	1-16 --- 17-32
X	Database GenBank, Accession No. AA148037, HILLIER et al., 'Generation and analysis of 280,000 human expressed sequence tags', abstract, 05 December 1996, Genome Res. Vol. 6, No. 9, pages 807-828, see sequence comparison.	1

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
15 DECEMBER 1999	4 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RAM R. SHUKLA Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20015

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CAMERON, E. R. Recent advances in transgenic technology. Molecular Biotechnology. 1997, Vol 7, pages 253-265, see entire document.	31-32

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20015

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A01K 67/00; A01N 61/00; G01N 33/53; C07K 14/00, 16/00; C07H 21/04; C12N 5/00, 15/63, 15/85, 15/86, 1/20, 15/00, 15/09; C12P 21/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

800/8; 514/1; 536/23.5; 435/320.1, 325, 252.1, 326, 6, 7.1, 375; 530/350, 387.1,